ARSENIC IN DRINKING WATER AND LOWER EXTREMITY ARTERIAL DISEASE IN BANGLADESH

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DECLARATION

I do hereby declare that this thesis entitled "ARSENIC IN DRINKING WATER AND LOWER EXTREMITY ARTERIAL DISEASE IN BANGLADESH" is based on the research work independently carried out by me. The research work was carried out at the Department of Occupational and Environmental Health, NIPSOM under the Faculty of Post-graduate Medical Science & Research, University of Dhaka, Dhaka, Bangladesh. All activities relating to this research and thesis has been carried out under the supervision of Prof. (Dr) Sk. Akhtar Ahmad, Professor and Head, Department of Occupational and Environmental Health, National Institute of Preventive and Social Medicine (NIPSOM), Mohakhali, Dhaka; and no part this thesis has been submitted elsewhere as the basis for the award of any degree or fellowship..

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CERTIFICATE

This thesis entitled "ARSENIC IN DRINKING WATER AND LOWER EXTREMITY ARTERIAL DISEASE IN BANGLADESH" has been submitted by Manzurul Haque Khan [Reg No. Session: 54/2009-2010 (Puna)] Asistant Professor, Dept of Occopational & Environmental Health, NIPSOM, in fulfillment of the requirements for the award of the Doctor of Philosophy (Ph.D) in Environmental Health under the Faculty of Post-graduate Medical Science & Research, University of Dhaka. All activities relating to this thesis has been carried out under my supervision. This thesis reports the findings of an independent research work and no part this thesis has been used as the basis for the award of any degree or fellowship in Bangladesh or elsewhere.

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DEDICATION

Dedicated to my ailing parents, and to all those suffering from health problems linked to arsenic exposure through drinking water in Bangladesh.

CONTENT

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Manzurul Haque Khan

ABSTRACT

This cross-sectional comparative study was carried out during the period 2004-2009 to assess if exposure to excess arsenic through drinking water posed any risk difference for lower extremity arterial disease (LEAD) in Bangladesh. A total of 3000 participants with equal numbers (1000) in each of the study groups [symptomatic exposure group (Gr I), asymptomatic exposure group (Gr II) and a reference group (Gr III)] all between the ages of 30-60 years were selected based on selected inclusion and exclusion criteria, and informed consent. During the process of selection of participants for Gr II and Gr III gender and age (±2 years) matching for participants recruited for Gr I was attempted. The Gr I participants had signs of arsenicosis (melanosis and/or keratosis) and history of consuming or having had consumed drinking water from sources identified as having excess arsenic (>0.05 mg/L). The Gr II participants had been selected from the household from which a participant for Gr I had been selected or from the neighboring household. Participants for Gr I and Gr II had been selected from Alamdanga, Shahrasti and Lakhsam upazillas while participants for Gr III had been selected from Pakundia upazilla. In the current study any participant having had amputation following gangrene or having a Doppler assisted ABI (ankle brachial index) of 0.90 or less was considered as having LEAD. For obtaining ABI, systolic pressures of both brachial arteries and both posterior tibial and dorsalis pedis arteries were obtained with the aid of Nicolet Vascular ABI kits with a 5 MHz Doppler probes, the higher of the two brachial pressures was taken as the brachial systolic pressure, and for each lower extremity, the higher of the two ankle systolic pressures (posterior tibial or dorsalis pedis arteries) was taken as the ankle systolic pressure. The ABI for each lower extremity was then calculated by dividing the ankle systolic pressure by the brachial systolic pressure. Any study participant having an ABI of 0.90 or less for any of the lower extremity was considered as having LEAD. Of the total 3000 study participants in three groups, one participant in Gr II had an ABI of >1.30 which implies that the particular participant had incompressible artery, and hence was excluded included from analyses. Thus the analysis was carried out on 2999 participants. Crude and adjusted prevalence rates for LEAD in each of the study groups were obtained along with their 95% confidence

interval (CI) and were compared for difference between the groups. To assess the differences in risk the crude odds ratios (ORs) and adjusted ORs along with their 95% CI were obtained through the binary logistic regression procedure. Dose-effect relationship between arsenic exposure through drinking water in Bangladesh and LEAD was also explored.

The study groups were statistically similar ($p>0.05$) in terms of gender, age, schooling, occupation, household size, household income, and type of house in which they lived. But the groups were found to be statistically different ($p<0.05$) in terms of household possession of agricultural land; household possession of television, radio or 2-in-1, bicycle and motorcycle. The study groups were statistically similar ($p>0.05$) in terms nutritional status (BMI) and smoking status (male). But were found to be statistically different ($p<0.05$) in terms of diabetic and blood pressure status of the participants. The crude prevalence of LEAD in Gr I, Gr II and Gr III were found to be 16.8%, 8.9% and 3.5% respectively, while the age and gender adjusted prevalence rates for participants in Gr I, Gr II and Gr III were found to be 16.8%, 9.2% and 3.3% respectively. Following adjustments for age, gender, obesity, diabetic status & HTN status, individuals having arsenicosis have 6.0 times (95% CI 4.1-8.8) higher of LEAD compared to individuals having background arsenic exposure (<0.05 mg/L) through drinking water; and on the other hand individuals not having arsenicosis but whose drinking water contains excess arsenic (>0.05mg/L) have a 2.8 times (95% CI 1.9-4.3) higher risk of LEAD compared to individuals having background arsenic exposure (<0.05 mg/L) through drinking water ($p>0.001$). A dose- effect relationship was observed between the prevalence of LEAD and arsenic exposure. The crude ORs (95% CI) of 4.1 (2.1-8.1), 4.9 (2.9-8.4) and 10.4 (6.1-17.5) for those who had cumulative arsenic exposure of 1-2, 5-9 and ≥10 mg/L-years respectively compared to those whose exposure level was <I mg/L-years; these ORs were found to be 3.0 (1.5- 6.0), 5.0 (2.9-8.7) and 7.7(4.5-13.2) when adjusted for age, gender, tobacco consumption, overweight/obesity, DM & HTN; and 3.0 (1.5-6.1), 5.7 (3.3-10.1) and 8.619 (4.9-15.1) when adjusted for all socio-demographic variables (age; gender; educational status; occupation; family ownership of agricultural land, radio television, bicycle, motorcycle; household size housing type; and annual household income); food habits (staple food type, numbers of days of vegetable intake per week,

number of days of fish/meat/egg intake per week, numbers of days of milk or milk products intake per week and cooking oil), tobacco consumption (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco), morbidity related variables (overweight or obesity, anaemia, pedal oedema, amputation, hypertension, diabetes). The finding of this study suggests a close relationship between long term arsenic exposure and lower extremity arterial disease in Bangladesh.

As individuals with LEAD besides suffering from the morbidity associated with progression of LEAD, have a profound increased risk of cardiovascular ischemic events and mortality compared to the general population. Arsenic exposed populations are also at risk at higher risk of cardiovascular ischemic events and mortality than the general population. Therefore it is urgent not only to enforce cessation of excess arsenic exposure but also to institute surveillance activities targeting LEAD and other atherosclerotic cardiovascular diseases and undertake actions to control or modify the modifiable cardiovascular risk factors for population exposed to excess arsenic in Bangladesh.

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ABBREVIATIONS

AD	Anno Domini
$(CH_3)_2AsOO^{\bullet}$	peroxyl radical
AB	Arsenobetaine
ABC	ATP-binding cassette transporter
ABC	ATP-binding cassette
ABI	Ankle brachial index
AC	Arsenocholine
AdoHcy	S-adenosylhomocysteine
AdoMet	S-adenosylmethionine
ADP	Adenosine diphosphate, adenosine-5′-diphosphate
APL	promyelocytic leukemia
AQP	aquaporins
As	Arsenic
As_2S_3	Orpiment
AS3MT	arsenite methyltransferase
As^{III}	Trivalent arsenic, arsenite
AsS	Realgar
As^{V}	Pentavalent arsenic, arsenate
Atm	Atmosphere
ATP	Adenosine triphosphate
B.C.	Before Christ
BFD	Blackfoot disease
CCA	Chromated copper arsenate
$CH_3As^{III}(OH)_2$, MAs^{III}	methylarsonous acid
DMA	Dimethylarsinic acid, Cacodylic acid
$DMAs^{V}$	dimethylarsenate
$DMAs^{III}$	dimethylarsinite
DSMA	disodium methylarsonate
Gal	Galectin 1
GLUT1	a mammalian glucose permease
GSH	Glutathione
GSSG	oxidized GSH

GST	glutathione-S-transferase
GST	GSH-S transferase
GST	Glutathione *S*-transferases
GSTO	Glutathione-S-transferase omega
H_2O_2	hydrogen peroxide
hAQP7 hAQP9	Human AQP
hGSTO-1	Human glutathione-S-transferase-omega
HXT	Hexose permease transporters
iAs^{III}	Inorganic arsenite, inorganic trivalent inorganic arsenic
iAs^{V}	Inorganic arsenate, inorganic pentavalent inorganic arsenic
LEAD	Lower Extremity Arterial Disease
LEPAD	Extremity Peripheral Arterial Disease
Ma	"millions of years ago" or megaannum
MDR/P-gp	multidrug-resistant/pglycoprotein
MORB	Mid-ocean Ridge Basalt
MMA	Methylarsonic acid, Monomethylarsonic acid
MAs^{III}	methylarsonite
MRP1, MRP2	Multidrug-resistant proteins I and 2
MSMA	monosodium methylarsonate
Na	Not available
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-kB,	Nuclear Factor -kappaB
NO	Nitric oxide, nitrogen monoxide
$O_2^{\cdot}$	superoxide anion radical
p450	Cytochrome p450
PAD	Peripheral Arterial Disease
PAOD	Peripheral arterial occlusive disease
PNP	purine nucleoside phosphorylase
ppb	Parts per billion
ppm	Parts per million
PVD	Peripheral Vascular Disease
ROS	reactive oxygen species
SAHC	S-adenosylhomocysteine

SAM	*S*-adenosylmethionine
TETRA	tetramethylarsonium ion
TMAO	trimethylarsine oxide
TR	thoredoxin reductase
W.H.O	World Health Organization
μg	micro gram

CHAPTER I

INTRODUCTION

1.1.0 INTRODUCTION

Lower Extremity Arterial Disease (LEAD) is a condition arising from chronic arterial narrowing that causes a mismatch between the oxygen supply and demand, resulting in symptoms of intermittent claudication, exercise limitations, and tissue loss ranging from ulceration to gangrene often culminating in spontaneous or surgical amputation(s).[1-12] Lower Extremity Arterial Disease is also known as Lower Extremity Atherosclerotic Occlusive Arterial Disease[3], Lower-limb arterial disease[5], Lower-Extremity Peripheral Arterial Disease (LEPAD)[3,9], Peripheral Vascular Disease (PVD)[4,10,13], Lower Extremity Peripheral Vascular Disease[9], Peripheral arterial occlusive disease (PAOD)[10] and Peripheral Arterial Disease (PAD)[1,3,5,7,8,11,14,15].

Lower Extremity Arterial Disease (LEAD) comprises a wide clinical spectrum ranging from individuals with asymptomatic arterial narrowing to those with the common symptom of intermittent claudication and at the severe end of the spectrum those with critical limb ischaemia (rest pain, ulceration, gangrene and spontaneous or surgical amputation). Symtomatic LEAD represents the tip of the iceberg of the disease burden in the population, as a substantially large proportion of the population have been found to have silent (asymptomatic) disease[1-5,7,14,16-23]. Asymptomatic disease has been found to be about three times as common as symptomatic disease[1-3,16,17,20,25]. LEAD is considered as a marker of atherosclerotic disease throughout the body[1-3,6,7,11,13,24,26]. Besides the morbidity associated with progression of LEAD, patients of LEAD have been found to have a profound increased risk of cardiovascular ischemic events and mortality compared to the general population[1-3,6-8,10,11,13,15,19-24,27-30]. Individuals having LEAD, whether symptomatic or asymptomatic, have been found to have an excessive risk of total and cardiovascular mortality[1-3,6-8,10,20,25,31].

Arsenic is a known carcinogen and has potentialities of producing cancers at multiple sites, notably in the skin, bladder, kidneys, prostate and lungs[33-38]. Arsenic is also known to have many non-cancer health effects that include peripheral vascular disease[32-51]. Vascular effects of arsenic have been reported over a span of more than 100 years[42]. Chronic ingestion of inorganic arsenic, notably from drinking water sources and arsenic contaminated wine or wine substitutes has been associated with the insidious development of peripheral vascular disease. An increased occurrence of gangrene in

Reichenstein, Silesia (now part of Poland) as result of arsenic exposure through drinking water has been reported back in 1898[43]. Peripheral arterial diseases leading to coldness of hands and feet, gangrene and amputations had been observed among vintners as a result of chronic arsenic exposure through consumption of contaminated wine[42]. An increased risk of LEAD as a result of drinking water containing arsenic has been observed in Taiwan, Mexico, Chile, the Xinjiang province of China and Thailand[44-48]. In Taiwan LEAD has been described as blackfoot disease (BFD), a peripheral vascular disease which clinically starts with numbness or coldness and ends with gangrene and spontaneous amputations of one or more of the affected extremities. Blackfoot disease is considered as an extreme manifestation of severe underlying systemic arteriosclerosis and the pathological types include arteriosclerosis obliterans (~70%) and thromboangitis obliterans (~30%)[37,38,41,42].

Many of the health problems known to be related to arsenic exposure including gangrene, are already evident amongst the arsenic exposed population in Bangladesh[39,52-69]. Though, gangrene which is considered as one of the extreme manifestations of LEAD has been reported in a number of studies focusing on the health effects of arsenic in Bangladesh[53,54,57,58]. Till date no published study is available that highlights the prevalence of LEAD amongst arsenic exposed population of Bangladesh. Against this perspective the current study had been designed to assess if exposure to excess arsenic through drinking water in Bangladesh possess any risk difference for LEAD.

Information gained from the study when implemented can be expected to help decide if there was any need to introduce surveillance program targeting LEAD amongst arsenic exposed population. And whether especial intervention program for prevention and modification of the progression of LEAD amongst arsenic exposed population in Bangladesh was required.

1.2.0 BACKGROUND INFORMATION

The word arsenic is derived from the Greek word *arsenikon*, meaning potent. Throughout the ages arsenic has been known for its killing properties. The word arsenic is surrounded by myth and mystery, and is often considered as a synonym for the word "toxic". Orpiment (As_2S_3), the bright yellow sulphide of arsenic had been named as "sandarach" by Aristotle in the 4th century B.C.[70,71,72]. Theophrastus called As_2S_3 by the name "arrenikon" while Discorides named it as "arsenicum". Orpiment was also named "auropigmentum" from the belief that it contained gold. In ancient Indian medical texts "Charaka-Samhita" the arsenic compounds orpiment (As_2S_3) and realgar (AsS) were named as "Ala or Haritala" and "Manahsila" respectively. Arsenic trioxide which is odorless and tasteless bears the name "king of poisons", which in France had earned the name "poudre de succession" or "inheritance powder". The multitude of names of white arsenic (arsenic trioxide) in Sanskrit (Sankh and Sabala Kshara), Hindi (Sanbul-Khar, Sammal khar, Sanbul and Sankyhia) and Bangla (Sanka or Senko) suggests general familiarity and extensive use of arsenical compounds. Although many alchemist claims to be the real discoverer of the metalloid arsenic, the discovery of elemental arsenic is generally credited to the German chemist Albertus Magnus (1193-1280) [70,71,73,74].

1.2.1.0 ARSENIC

Arsenic (As) is a metalloid that belongs to group 15 (old group V) of the periodic table. It's atomic number is 33 and it has an atomic weight of 74.92. In its elemental form, arsenic is a grayish metal. Pure arsenic (which rarely is found in nature) exists in three allotropic forms: yellow (alpha), black (beta), and grey (gamma); the grey form is the stable form. The metastable yellow form rapidly changes to the grey semi-metallic form at low temperatures, and instantaneously in sunlight at room temperature. Brown and black amorphous forms are also known. The grey form is a crystalline solid with a brilliant cluster. It is a good conductor of heat but a poor electrical conductor. Elemental arsenic has a specific gravity of 5.73, sublimes at 613°C, and in the absence of oxygen it melts at 817°C (28 atm). Arsenic has four valency states (-3, 0, +3 and +5), the valence state 0 refers to elemental arsenic. Arsenic and arsenic compounds occur in crystalline, powder, amorphous, or vitreous forms. Many of the inorganic arsenic compounds occur

as white, odorless solids with specific gravities ranging from about 1.9 to more than 5. In strongly reducing environments, elemental arsenic (0) and arsine (–3) can exist. Under moderately reducing conditions, arsenite (+3) may be the dominant form, but arsenate (+5) is generally the stable oxidation state in oxygenated environments[32,33,36,37,41, 71-75].

1.2.2.0 ARSENIC COMPOUNDS: MINEROLOGICAL & CHEMICAL FORMS[32,-34, 37,41,44,70,71,72,74,76,77]

Table-1: Arsenic containing naturally occurring minerals.

Mineral	Formula	Mineral	Formula
Adamite	$Zn_2AsO_4((OH)$	Nicolite	NiAs
Annabergite	$N_{13}(AsO_4)_2.\ 8H_2O$	Olivenite	Cu_2OHAsO_4
Antimony arsenide	AsSb	Orpiment	As_2S_3
Arsenolite	As_2O_3	Pearcite	$Ag_{16}As_2S_{12}$
Arsenopyrite	FeAsS	Pharmacosiderite	$Fe_3(AsO_4)_2OH_3$
Beaudanite	$PbFe_3(AsO_4)SO_4$	Proustite	Ag_2AsS_3
Cobaltite	CoAsS	Rathite	$Pb_3As_5S_{10}$
Energite	Cu_3AsS_4	Realgar	As_4S_4 AsS
Erythrite	$Co_3AsO_4.8H_2O$	Scorodite	$(Fe.Al)AsO_4.2H_2O$
Gersdorffite	CoAsS	Skutterudite	$(Co,Ni,Fe)As_3$
Jordanite	$(PbTi)_{13}As_7S_{23}$	Smaltite	$(Co,Fe,Ni)As_2$
Loellingite	$FeAs_2$	Tennantite	$Cu_{12}As_4S_{13}$
Mutite	$Pb_5(PO_4.AsO_4)_3Cl$		

Many inorganic arsenic compounds are found in the environment, frequently occurring as the sulfide form in complex minerals containing copper, lead, iron, nickel, cobalt, and other metals. Arsenic is the main constituent of more than 245 mineral species, of which about 60% are arsenate, 20% sulfide and sulfosalts and the remaining 20% include arsenides, arsenites, oxides and elemental arsenic. The highest mineral concentrations generally occur as arsenides of copper, lead, silver, or gold or as the sulfide. Naturally occurring arsenic bearing minerals are listed in table-1. Important arsenic minerals include arsenopyrite (FeAsS), realgar (As_4S_4), orpiment (As_2S_3), domeykite (Cu_3As),

loellingite ($FeAs_2$), nicolite (NiAs), cobalt glance (CoAsS), nickel glance (NiAsS), smaltite ($CoAs_2$) and arsenolite (As_2O_3) . Some other important arsenic compounds are listed in table-2. The name arsenic usually refers to arsenic trioxide (As_2O_3), rather than

Table- 2: - Some important arsenic compounds.

Inorganic trivalent arsenic compounds	
Arsenic(III) oxide	As_2O_3
(Arsenic trioxide, arsenous oxide, white arsenic)	
Arsenous acid (arsenenous acid, arsenious acid)	H_3AsO_3, $HasO_3^{-2}$, $H_2AsO_3^{-}$, AsO_3^{-3},
Arsenic trichloride (Arsenous trichloride)	$AsCl_3$
Arsenic sulphide	As_2S_3
(arsenic trisulfide, orpiment, auripigment)	
Inorganic pentavalent arsenic compounds	
Arsenic(IV) oxide (arsenic pentoxide)	As_2O_5
Arsenic acid (Orthoarsenic acid)	H_3AsO_4, $HAsO_3$, $H_2AsO_4^{-}$, $HasO_4^{-2}$, AsO_4^{-3}
Arsenenic acid (meta-arsenic acid)	
Arsine	AsH_3
Organic arsenic compounds	
Methylarsonic acid	$CH_3AsO(OH)_2$
(methanearsonic acid, MMA)	
Dimethylarsinic acid (Cacodylic acid, DMA)	$(CH_3)_2AsO(OH)$
Trimethylarsine oxide	$(CH_3)_3AsO$
Arsanilic acid (p-aminobenzene arsonic acid)	
Arsphenamine	
(2-aminophenol dihydrochloride, Salvarsan)	
Carbarsone	
(phenylarsonic acid, N-carbmoylarsanilic acid)	
Tryparsamide	
[4-[2-amino-2-oxoethyl)amino]-phenyl]arsonic acid	
Arsenobetaine	$(CH_3)_3As^{+}CH_2COOH$
Arsenocholine	$(CH_3)_3As+CH_2CH_2OH$
Dialkylcholoroarsine	
Alkyldichloroarsine	
dimethylarsinoylribosides	
trialkylarsonioribosides	
dimethylarsinoylribitol sulfate	

to arsenic as an element. Oxides of arsenic (arsenic trioxide and arsenic pentoxide) and acids of arsenic (arsenous and arsenic acids) in environment are the weathering products of different mineralogical forms and compounds of arsenic. Arsenic trioxide, the most common arsenic compound in commerce, melts at 315°C and boils at 465°C (table -3).

Table- 3: - Some important inorganic compounds.

Arsenic compound	Boiling point (°C)	Melting point (°C)
INORGANIC (III)		
Arsenic sulphide	707	300 to 325
Arsenic trichloride	130	-16
Arsenic trioxide	465	315
Cupric arsenite	n.a.	Decomposes
Gallium arsenide	n.a.	1238
Potassium arsenite	63.09	300 (decomposes)
Sodium arsenite	97.8	615
INORGANIC (V)		
Arsenic pentoxide	n.a.	315 (decomposes)
Calcium arsenate	n.a.	1455 (decomposes)
Lead arsenate	n.a.	280 (decomposes)
Potassium arsenate	n.a.	288
Sodium arsenate	180 (decomposes)	130

Arsenic trioxide (As_2O_3) is the primary product of arsenic smelters. It is also the weathering product of all arsenic minerals. It is used for preparing other commercially important inorganic and organic arsenic compounds. It has 3 allotropic forms: the cubic form- Arsenolite which has a melting point 227°C, the monoclinic Claudetite that melts at 313°C, and takes an amorphous glassy form. Arsenic trioxide has a boiling point of 457-465 °C. When As_2O_3 is dissolved in water, the water contains o-arsenous acid (H_3AsO_3), in acidic solutions only the undissociated species $As(OH)_3$ is detected, while in alkaline solutions the $H_2AsO_3^-$, $HAsO_3^{-2}$, AsO_3^{-3} anions are found. The aqueous solution has a sweet metallic taste. Arsenic pentoxide (As_2O_5) is white, crystalline and hygroscopic. Trivalent arsenic is more toxic than pentavalent arsenic. In this text the word "arsenic" refers to arsenical compounds in general unless otherwise stated.

1.2.3.0 SOLUBILITY OF ARSENIC COMPOUNDS[33 37,71,75-79]

The alkali-metal arsenite are freely soluble in water, the alkaline-earth arsenites are slightly soluble, and the heavy- metal arsenites are insoluble. Arsenic sulphides have low solubility. In water, elemental arsenic is insoluble, calcium arsenate and arsenites are sparingly soluble, and arsenic trioxide, arsenic pentoxide, and other arsenicals are soluble. As_2O_3 is slightly soluble in water. The solubility of arsenic trioxide in 100g of water is 1.2g at 0° C, 2.1g at 25° C and 5.6g at 75° C. As_2O_5 is highly soluble in water, as much as 630g of arsenic pentoxide can be dissolved in 100g of water.

Arsenic pentoxide, potassium arsenite, and the sodium salts are soluble in ethanol. Arsenic, arsenic pentoxide, arsenic trioxide, the calcium arsenites, lead arsenate, and potassium arsenate are soluble in various acids. When heated to decomposition, arsenic compounds emit arsenic fumes. The solubility of some common arsenic compounds are listed in table-4.

Table -4 : Arsenic and salts: physico-chemical properties

Arsenic compound	Normal state (at room temperature)	Colour	Odour	Soluble (g/litre)	Insoluble
INORGANIC (III)					
Arsenic sulphide As_2S_3	Powder	Yellow-red	n.a.	Hot water (slightly) Alkali, Acids Ethanol	Water (cold)
Arsenic trichloride $AsCl_3$	Liquid (oily)	Colourless	Acrid smell	Ethanol Ether Concentrated mineral acids	decomposed by water
Arsenic trioxide As_2O_3	Powder amorphous or crystalline	White	Odourless	Water-cold (12) Water-hot (115) Alkali, HCl	Alcohol Chloroform Ether
Cupric arsenite $CuHAsO_3$	Powder	Yellowish green	n.a	Acids Ammonia	Water Alcohol
Gallium arsenide AsGa	Solid (cubic crystals)	Dark grey with metallic sheen	n.a.	n.a.	Water
Potassium arsenite $KH(AsO_2)_2$	Powder	White	n.a.	Water Ethanol (Slightly)	n.a.
Sodium arsenite $NaAsO_2$	Powder	White or greyish- white	n.a.	Water (very) Ethyl alcohol (slightly)	n.a.
INORGANIC (V)					
Arsenic pentoxide As_2O_5	Powder (hygroscopic)	White	n.a.	Cold water (1500) Hot water (767)	n.a.
Calcium arsenate $Ca_3(AsO_4)_2$	Powder (amorphous)	Colourless	Odourless	Water (slightly) Dilute acids	n.a.
Lead arsenate $PbHAsO_4$	Powder or solid (crystalline)	White	n.a.	Hot water (slightly) Dilute nitric acid Caustic alkalis	n.a.
Potassium arsenate KH_2AsO_4	Powder (crystalline)	White	n.a.	Cold water (190) Hot water (very) Glycerol Ammonia	n.a.
Sodium arsenate Na_3AsO_4	Powder	Clear colourless	Odourless	Water (very) alcohol (slightly)	Ether

n.a.: not available

1.2.4.0 ARSENIC IN NATURE

Arsenic is ubiquitous in nature. It is present in varying amounts in different environmental components. It has been estimated that of the total arsenic contained in the various reservoirs (rocks, oceans, soils, biota, atmosphere), more than 99% is associated with rocks and minerals. Arsenic ranks 20th in abundance in the earth's crust, 14th in sea water, 12th in human body [71, 80].

Arsenic in rocks and Earth's crust[33,34,37, 73,74,79-83]

Arsenic ranks 20th among the elements in abundance in the earth's crust. The total amount of arsenic in the upper earth crust is estimated to be 4.01 x 10^{16} kg. The abundance in the continental crust is about 1.5 to 2 ppm. The average arsenic content of igneous rocks varies from 1.5 to 3.0 ppm (1.0 to 5.9 mg/kg), while in sedimentary rocks it ranges from 1.7 to 400 ppm (5 to 10 mg/kg). Volcanic glasses have a higher average arsenic content which is around 5.9 mg/kg (2.2-12.2 mg/kg). Shales, clays, phosphate rocks, and sedimentary iron and manganese oxides are generally enriched in arsenic. Most of the arsenic in non marine clays and shales is associated with the clay minerals. Coal often contains appreciable quantity of arsenic.

Table No- 5: Typical Arsenic Concentrations in Rock-Forming Minerals[83,142].

Mineral	Arsenic concentration (mg/kg)	Mineral	Arsenic concentration (mg/kg)	Mineral	Arsenic concentration (mg/kg)
Sulfide minerals		**Silicate minerals**		**Sulfate minerals**	
Pyrite	100–77,000	Quartz	***0.4–1.3***	Gypsum /anhydrite	<1–6
Pyrrhotite	5–100	Feldspar	***<0.1–2.1***	Barite	<1–12
Marcasite	20–126,000	Biotite	***1.4***	Jarosite	34–1,000
Galena	5–10,000	Amphibole	***1.1–2.3***	**Other minerals**	
Sphalerite	5–17,000	Olivine	***0.08–0.17***	Apatite	<1–1,000
Chalcopyrite	10–5,000	Pyroxene	***0.05–0.8***	Halite	<3–30
Oxide minerals		**Carbonate minerals**		Fluorite	<2
Hematite	up to 160	Calcite	***1–8***		
Fe oxide (undifferentiated)	up to 2,000	Dolomite	***<3***		
Fe(III) oxyhydroxide	up to 76,000	Siderite	***<3***		
Magnetite	2.7–41				
Ilmenite	<1				

Arsenic in air[33,34,37,73,74,79-85]

Generally very low amounts of arsenic may be found in air. But it may be increased by inputs from smelting and other industrial operations, fossil-fuel combustion, microbial reduction and low temperature volatilization (mainly biological formation of volatile arsenicals) and volcanic activity. Much of the atmospheric arsenic is particulate. Arsine and methylated arsines ($MeAsH_2$, Me_2AsH, Me_3As) have been reported over sites where biological activity is high. These species then partly oxidizes to the pentavalent state. An estimated 8.12 kilotons of arsenic occur in the atmosphere. Concentrations amounting to around 10^{-5} to10^{-3} μg^{-3} have been recorded in unpolluted areas, increasing to 0.003–0.18 μg^{-3} in urban areas and greater than 1 $\mu g\ m^{-3}$ close to industrial plants.

Total atmospheric arsenic emissions from both natural and anthropogenic sources have been estimated to be 31×10^9 g/year, and total atmospheric arsenic removal was estimated to be 30~50 $\times 10^9$ g/year. The global tropospheric residence time of arsenic appears to be about 9 days. Total emissions were estimated at 36×10^9 g/year, with the major source of atmospheric arsenic being anthropogenic emissions; the major natural source of arsenic was volcanic activity. Emissions from anthropogenic sources were estimated at 24 $\times 10^9$g/year, representing 64% of total arsenic influxes. Depositions from the atmosphere to the land and the oceans were estimated at 24×10^9 g/year and 9×10^9 g/year respectively. Total arsenic deposition rates have been calculated to be in the range of <1-1000μg^{-2}/yr, depending on the relative proportions of wet and dry deposition, and proximity to contamination sources. The atmospheric residence time of particulate-bound arsenic depends on particle size and meteorological conditions, but a typical value is about 9 days.

Arsenic in soil[33,34,37,73,73,79-85]

The dominant source of arsenic in soils is geological, and hence dependent to some extent on the concentration in the parent rock material. Arsenic may also accumulate in soil through anthropogenic sources such as pesticides, herbicides, fungicides, fertilizers (especially phosphate fertilizers), irrigation water from mines, dust from fossil fuel combustion and disposal of industrial (smelting) and animal wastes.

Table No- 6 : Typical Arsenic Concentration Ranges in Rocks, Sediments, and Soils

Classification	Rocks/ Sediments type	Arsenic Concentration Ranges (mg/kg)
Igneous rocks	Ultrabasic rocks	0.03–16
	Basic rocks	1.5–110
	Intermediate	0.09–13
	Acidic rocks	0.2–15
	Volcanic glasses	2.2-12.2
Metamorphic rocks	Quartzite	2.2–7.6
	Hornfels	0.7–11
	Phyllite/slate	0.5–140
	Schist/gneiss	<0.1–19
	Amphibolite/greenstone	0.4–45
Sedimentary rocks	Shale/mudstone	3–490
	Sandstone	0.6–120
	Limestone	0.1–20
	Phosphorite	0.4–190
	Iron formations and iron-rich	1–2,900
	sediment	0.1–10
	Evaporite deposits	0.3–35,000
	Coal	100–900
	Bituminous shale	
Unconsolidated Sediments and soils	Sediments	0.5–50
	Soils	0.1–55
	Soils near sulfide deposits	2–8,000

Arsenic in soil as unweathered sulphide minerals or in an inorganic anion state, arsenopyrite is the most commonly occurring compound in soil, but arsenosulfides of any metal cation may be found. Inorganic arsenates bound to iron an aluminum cations and oxides, it may also be found in association with cations like calcium, magnesium, lead and zinc. Arsenic is also found bound to organic matter in soil.

Baseline concentrations of arsenic in soils are generally of the order of 5–10 mg/ kg. Peats and bog soils can have higher concentrations (average 13 mg/kg), principally because of increased prevalence of sulphide mineral phases under the reduced conditions. Acid sulphate soils which are generated by the oxidation of pyrite in sulphide-rich terrains such as pyrite-rich shales, mineral veins and dewatered mangrove swamps can also be relatively enriched in arsenic. Arsenic occurs mainly as inorganic species, with depending on the pH and the redox conditions of the soil, arsenates and arsenites being the stable arsenic species. Inorganic arsenic compounds can be methylated to MMA,

DMA and TMAO by microorganisms. Further reduction can lead to volatile trivalent methylarsines. As^{V} , As^{III}, MMA and DMA are the predominant species.

An arsenic loss of 17–35% per year from soil occurs through volatilization and it is directly related to the nutrient levels and microbial growth in soil. Leaching does not appear to be a significant route of arsenic loss from soil. Movement of arsenic to depths is significantly greater in a sandy soil than in clay soils. Depending on soil characteristics arsenic could usually leach to depths in between 30 to 40 cm, and rarely to depths of ~120 cm. Approximately 7% of the amount applied would be transported from the watershed by runoff and erosion, 38% in solution and 62% attached to sediment.

The calculated average half-life of arsenic (arsenite) persistence in soil is about 6.5 ± 0.4 years. Arsenic is found in all soils and the geologic history of a particular soil is considered to be the determinant for arsenic content of virgin soil. The arsenic content in virgin soil generally varies from 0.1 to 40 ppm. The average arsenic content of soils is about 5 to 6 ppm. Peaty and bog soils, and soils near sulfide deposits can have higher concentration of arsenic.

Arsenic in biota[33,34,37,73,74,79-85]

Arsenic is the 12th most abundant element in the biosphere. In freshwater organisms and terrestrial biota arsenic levels are generally less than 1 mg/kg. In terrestrial biota wide variations in levels of arsenic may occur depending on the species and the arsenic concentration in the growing environment of the biota. On the basis of total wet weight average arsenic content of freshwater fish is 0.54mg/kg, bottom feeding organisms tend to have higher levels of arsenic.

Arsenic levels in terrestrial plants are generally lower than those found in marine plants. The concentrations seldom exceed levels higher than 1 mg/kg dry mass. In plants inorganic arsenic predominates (arsenite, or more usually arsenate), MMA (methylarsonic acid) and DMA (dimethylarsinic acid) can be found as well. TMAO (trimethylarsine oxide), TETRA (tetramethylarsonium ion), AB (arsenobetaine) and presumably also AC (arsenocholine) have also been detected. In freshwater plants arsenite and arsenate predominate and low levels of MA and DMA are also detectable.

Arsenosugars are only occasionally detected. TETRA and an appreciable amount of lipid-soluble arsenic can be found as well.

In seaweeds (macroalgae) high levels of arsenic (up to 230 mg/kg dry mass) occur. Arsenosugars (dimethylarsinoyl or a trialkylarsonium derivative bound to a ribofuranoside sugar) are the principal form of arsenic found in seaweed. A total of 17 different species of natural arsenosugars have been isolated; of them 13 are dimethylarsinoyl derivatives, 4 are quaternary arsonio analogues and among them 12 are water-soluble and only one compound is lipid-soluble.

In terrestrial mammals arsenic accumulates specifically in ectodermal tissues. Domestic animals and human generally contain less than 0.3μg/g (wet weight). Total arsenic content of human body varies between 3 to 4 mg and it tends to increase with age. With the exception of hair, nail and teeth most body tissue contains less than 0.3 to 147 μg/g arsenic on the basis of dry weight.

In marine environment the concentrations of arsenosugars in brown algae (<1-230 mg/kg dry mass) are higher than those found in red (0.4-45 mg/kg dry mass) and green (0.1-23 mg/kg dry mass) species. Other commonly found minor arsenic constituent in macroalgae include DMA. As^{III}, As^{V} and MA may occur in trace amounts. AB appears to be the major arsenical in marine animals. TMAO has been found as a minor natural component, and AC appears only at trace amounts. In marine animals TMAO and AC are considered as a breakdown product and a precursor of AB. AB is now generally accepted as non-toxic. TETRA is found in considerable concentrations in many crustacean and mollusc species, although marine animals contain many arsenic compounds but AB is the major arsenical. Fish tend to have a simple pattern of arsenic compounds dominated by AB. Crustaceans generally contain AB as a high percentage of their total arsenic. Gastropods often contain very high arsenic concentrations (up to 340 mg/kg wet mass), mostly in the form of AB. Bivalve molluscs in addition to AB, contain large quantities of TETRA. Some scallop species contain high levels of arsenosugar. Marine mammals contain only low levels of arsenic. In all cases AB predominates. Lipid arsenic compounds also occur in marine animals. MMA, DMA, inorganic arsenic and some unknown species of arsenic appear at trace or minor levels in marine animals as well.

Arsenic in river, lake and sea water[33,34,37,71,73,74,79-85]

Arsenic concentration in natural water varies depending on the source of water, source of arsenic and local conditions. Arsenic concentration in unpolluted river water is usually low, between 1 to 10µg/L, but is higher in polluted rivers. They vary according to the composition of the surface recharge, the contribution from base-flow and the bedrock lithology. Concentrations at the low end of the range have been found in rivers draining arsenic poor bedrocks. Relatively high concentrations of naturally occurring arsenic can occur in some areas, as a result of inputs from geothermal sources or high-arsenic groundwaters.

Increased concentrations have been reported in some river waters from arid areas where the surface water is dominated by river base-flow, i.e. by water flowing into the surface water from the surrounding rocks. Significant increases in arsenic concentrations of river waters may also occur as a result of pollution from industrial effluents or sewage. Arsenic can also be derived from mine wastes and mill tailings. High arsenic concentrations (typically around 200–300 µg/L) are found in surface waters affected respectively by tin and gold mining activities.

Arsenic concentration in lake water is usually close to or lower than that generally found in river water. High concentrations may be found in water of alkaline closed basin lakes due to high evaporation and geothermal inputs.

An estimated 3.7×10^6 ktons of arsenic occur in the oceans. In seawater Arsenic content is relatively constant around 1.5µg/L. Concentrations in estuarine water are more variable as a result of varying river inputs and salinity or redox gradients, but are also usually low at typically less than 4 µg/L under natural conditions. Concentrations are commonly higher when riverine inputs are affected by industrial or mining effluent or by geothermal water.

In oxic seawater arsenic is typically dominated by As^{V}, though some As^{III} is invariably present and becomes of increasing importance in anoxic bottom waters. Ratios of As^{V}/As^{III} are typically in the range 10–100 in open seawater. Arsenic^{V} exists mainly as $HAsO_4^{2-}$ and H_2AsO_4 and As^{III} exist mainly as the neutral species H_3AsO_3.

Relatively high proportions of H_3AsO_3 are found in surface waters. Increases in organic arsenic species occur as result of methylation reactions by phytoplankton. The relative

proportions of arsenic species are more variable in estuarine waters because of variable redox and salinity, and terrestrial inputs. However, they are dominated by As^{V}.

In marine and estuarine waters, organic forms are usually less abundant but are nonetheless often detected. Concentrations of these depend on abundance and species of biota present and on temperature.

In lake and river waters, As^{V} is generally the dominant species, significant seasonal variations in speciation as well as absolute concentration may occur. Concentrations and relative proportions of As^{V} and As^{III} vary according to changes in input sources, redox conditions and biological activity. The presence of As^{III} may be maintained in oxic waters by biological reduction of As^{V}, particularly during summer months. Higher relative proportions of As^{III} may be found in river stretches close to inputs of As^{III} dominated industrial effluent and in waters with a component of geothermal water. Organic forms of arsenic are usually minor in surface waters. Nonetheless, proportions of organic forms of arsenic can increase as a result of methylation reactions catalysed by microbial activity (bacteria, yeasts, algae). The dominant organic forms found are dimethylarsinic acid (DMAA; $(CH_3)_2AsO(OH)$) and monomethylarsonic acid (MMAA; $CH_3AsO(OH)_2$), where arsenic is present in both cases in the pentavalent oxidation state. Proportions of these two species have been noted to increase in summer as a result of increased microbial activity. The organic species may also be more prevalent close to the sediment-water interface.

Arsenic in sediments[33,34,37,71,73,74,79-85]

Concentrations of arsenic in unconsolidated sediments are not notably different from those in their indurated equivalents, muds and clays having typically higher concentrations than sands and carbonates. Values are typically 3–10 mg/kg, depending on texture and mineralogy. An estimated 25 $x10^{9}$ ktons of arsenic occur in sediments. Elevated concentrations tend to reflect the amounts of pyrite or iron oxides present. *Placer* deposits in streams can have very high concentrations as a result of the abundance of sulphide minerals. An average arsenic concentration for stream sediments of England and Wales has been found in the range of 5-8 mg/kg. average arsenic concentrations in sediments has been reported to be 2.0 mg/kg (range 1.2–2.6 mg/kg) for the River Ganges, 2.8 mg/kg (range 1.4–5.9 mg/kg) for the Brahmaputra River and 3.5 mg/kg (range 1.3–

5.6 mg/kg) for the Meghna River. The average level of arsenic in marine sediments of the world oceans is about 40 mg/kg. Sediments from coastal regions and estuaries contain rather lower concentrations of arsenic than deep-sea sediments. Relative arsenic enrichments have been observed in reducing sediments in both nearshore and continental-shelf deposits. Concentrations increase with depth (up to 30 cm) in continental shelf sediments as a result of the generation of increasingly reducing conditions. Concentrations varied between sites, but generally increased with depth in the range 2.3–8.2 mg/ kg.

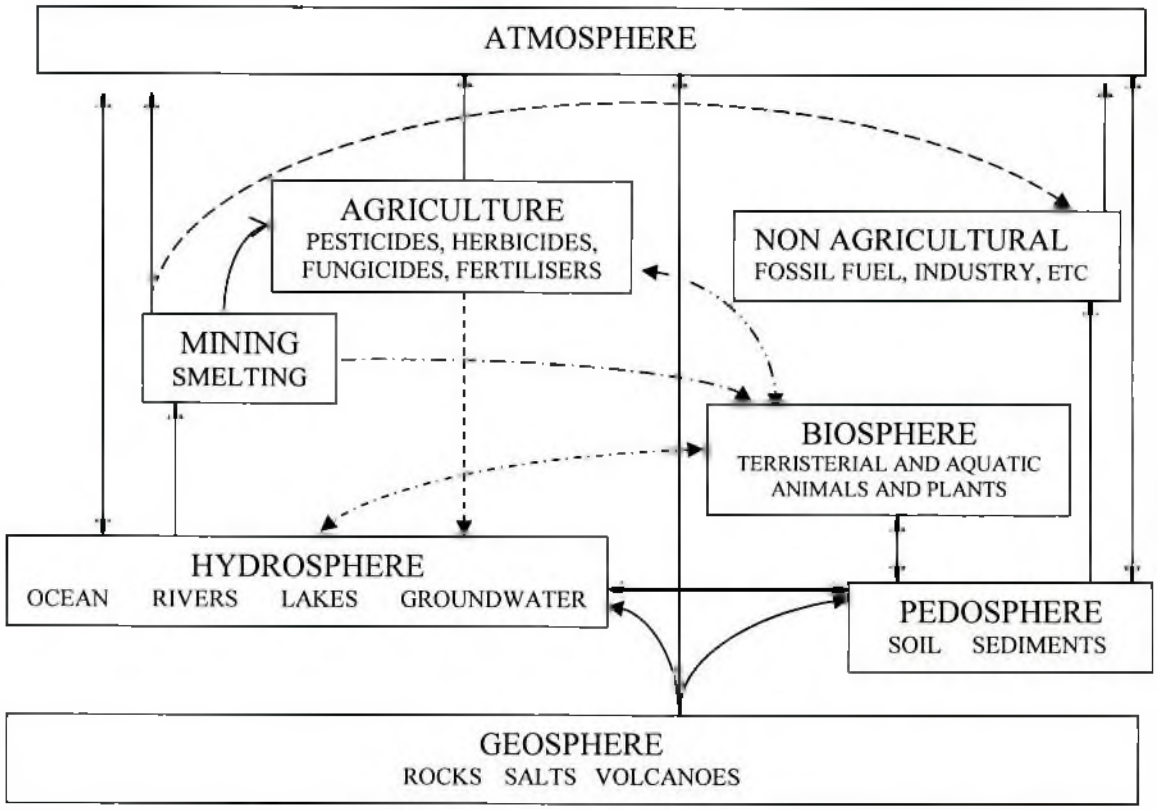
Arsenic in groundwater[33,34,37,71,73,74,79-85]

Under natural conditions, the greatest range and the highest concentrations of arsenic are found in groundwaters as a result of the strong influence of water-rock interactions and the greater tendency in aquifers for the physical and geochemical conditions to be favorable for arsenic mobilization and accumulation. High concentrations of arsenic are found in groundwater in a variety of environments. This includes both oxidizing and reducing aquifers and in areas affected by geothermal, mining and industrial activity. Most high-arsenic groundwater instances are the result of natural occurrences of arsenic. Cases of mining-induced arsenic pollution are numerous but tend to be localized. Cases of industrially-induced arsenic pollution (including that from agriculture) may be severe locally, but such occurrences are relatively rare. In general, the background concentrations of arsenic in groundwater in most countries are less than 10 μg/L or sometimes substantially lower, the arsenic in ground water may vary in a large range from <0.5–5000 μg/L. In areas with volcanic rock and sulphide mineral deposits arsenic levels in ground water can range up to 3.4 mg/L. In groundwaters, the ratio of As^{III} to As^{V} can vary enormously as a result of large variations in aquifer redox conditions, redox gradients and history. In strongly reducing aquifers (Fe^{III}- and sulphate-reducing), As^{III} typically dominates. Concentrations of organic forms are generally negligible in ground waters.

1.2.5.0 ARSENIC RECYCLING [33,37,71,73,74,78,79-86]

The arsenic found in geosphere, biosphere, hydrosphere, pedosphere and atmosphere is transferred from one compartment to another by natural or anthropogenic processes (figure-1).

Figure-1: Environmental cycling of arsenic.



Rocks and minerals are the main reservoirs of arsenic. Natural weathering processes, volcanic activity, geothermal activity, biological activity and anthropogenic activities (including burning of vegetations). Arsenic enters the atmosphere through inputs from wind erosion, volcanic emissions, low temperature volatilisation from soils, marine aerosols and pollution and is returned to the earth's surface by wet and dry deposition. Anthropogenic sources have an important impact on airborne arsenic compositions. The most important anthropogenic inputs are from smelter operations and fossil-fuel combustion. Electrical generators burning soft brown coal can also contaminate the air.

The arsenic appears to consist of mainly As_2O_3 dust particles. Anthropogenic sources contribute to about 30% (around 18,800 tons yr^{-1}) of the global atmospheric arsenic flux. Baseline concentrations of arsenic in rainfall and snow in rural areas are invariably low at typically less than 0.03 μg/L. Concentrations in areas affected by smelter operations, coal burning and volcanic emissions are generally higher. Atmospheric precipitation contributes some arsenic to land and to surface and groundwater bodies.

Weathering of rock converts arsenic rich metal sulfides to arsenic trioxide which eventually finds its way into surface water, groundwater and sediments. Significant increases in arsenic concentrations of river waters may also occur as a result of pollution from industrial or sewage effluents. Arsenic can also be derived from mine wastes and mill tailings.

Arsenic is often found associated with iron oxyhydroxides in sediments. The oxidized forms of arsenic are often converted back to sulphides by anaerobic processes occurring on land and in sediments. Volatile forms of arsenic (arsine and trimethyl arsine) enter the atmosphere from land and water and are returned by rain and atmospheric fallout.

Use of arsenical pesticides, herbicides, fungicides, fertilizers and other arsenic compounds results in build up of arsenic in soil form where it often finds its way to water bodies. In addition some of this arsenic is transformed to methylated volatile arsenic forms which eventually go up into the atmosphere.

1.2.6.0 USES OF ARSENIC

Historically, arsenic (As) is well known for its killing properties. The word arsenic in the general vocabulary is used as a synonym for 'toxic' and is surrounded by mystery and myth. The use of arsenic dates back to the Copper age, if not to an earlier time. Arsenic though not found in Egypt, has been in use in ancient Egypt (orpiment has been found in a linen bag in King Tutankhamen's tomb and in the wall paintings of the Theban necropolis) indicating it's trade at least as early as 1322 B.C. Toxic properties of arsenic were known even as early as 340 B.C., which is evident from Aristotle's remark "Sandarach kills horses as well as any kind of draught cattle"[70,73,78, 87,88].

1.2.6.1 NON MEDICINAL USES[70,73, 87,88,89]

During this period arsenical-copper alloys were produced often containing appreciable quantities of arsenic (0.25 to 12%). Civilizations from those of the Tape Yahya in Iran (4th millennium B.C.) to the pre-Columbian Chimu artisans of the Central Andes valued the properties of the copper arsenic alloy. Arsenic was used in the 3rd millennium to produce a silvery surface effect on mirrors and animal statuettes and as a fluxing ingredient in the manufacture of glass. In the Middle Ages the yellow sulfide of arsenic known as auropigmentum was used as a common ingredient for most colors used by painters. Pliny (23-79 A.D.) described arsenical preparations as poisons and pigments. Peasants of Styrian Alps of Austria in the 19th century habitually used to consume arsenic as a means for promoting physical stamina. In the nineteenth century arsenicals were ingested, inhaled as vapours, injected intramuscularly and intravenously, and given in enemas. They caused cutaneous capillary dilatation - the fashionable 'milk and roses' complexion. Arsenic has been used as a poison for homicidal purpose especially in the Middle Ages, and in the manufacture of war gases (lewisite).

Arsenic and agriculture[32,33,37,70,73,74]

The Chinese Encyclopedia (1600) because of it's toxic properties has recommended it's use against insects in rice crops. Since 1860s inorganic compounds of arsenic had been the dominant insecticide used by farmers and fruit growers, till the introduction of DDT and other organic pesticides in 1940s. Organic arsenic compounds like monosodium methylarsonate (MSMA), disodium methylarsonate (DSMA), dimethylarsinic acid (cacodylic acid) are still in use. Arsenic acid is still used for formation of wood preservative salts. Arsenic is also used as defoliant, soil sterilant and for debarking trees.

Arsenic and livestock[32,33,37,70,73,74,83]

Arsenilic acid is used as an additive in poultry, swine, and veterinary feed. It is used in cattle and sheep dips; and as algaecides. It is also used for prevention of diseases like swine dysentery and heartworm infection.

Arsenic in metallurgy and industries[32,33,37,70,73,74]

Metallic arsenic is mainly used for making alloys. It is used in making lead-antimony grid alloys for acid batteries. It is used to harden the lead-base bearing alloys of internal

combustion engines, in making automotive body solders and lead ammunition. Arsenic is used to raise the recrystallization temperature and improvement of corrosion resistance of copper and copper alloys. Arsenic containing alloys are used in making locomotive fireboxes, condenser tubes, heat exchangers and distillation tubes.

High purity arsenic is used for making low melting glasses and semiconductor compounds used in light emitting diodes, solar cells, infrared emitters and devices, etc.

It is also used in the preparation of catalysts, pyrotechnics, antifouling paint, dyes and soaps, ceramics and in electro-photography.

1.2.6.2 MEDICINAL USE OF ARSENIC [33,41,70,73,74,78,85-102]

Arsenic compounds have a long history of use in medicine. It has been used both as therapeutic agent and as poison for more than 2,400 years. Some medicinal uses are listed in table-7. Arsenic compounds were valued by ancient Indian cultures during the ages of Buddha. The Charaka-Samahita medical text recommended *Ala* or *Haritala* (orpiment) and *Manashila* (realgar) for external and internal medication. *Hippocrates* used the arsenic sulfides realgar and orpiment to treat ulcers, and *Dioscorides* used the latter as a depilatory. Fowler's solution (1% potassium arsenite, discovered in 1786) became the most widely used medication for a variety of illnesses for 150 years. It was used empirically for the treatment of a variety of diseases including asthma, chorea, eczema, pemphigus, and psoriasis.

Following its introduction, Fowler's solution became a standard remedy to treat anemia, Hodgkin's disease, and leukemia. Many arsenic preparations were used therapeutically in the 18th century. In the 19th century, arsenic was the mainstay of the Materia Medica. Although pure metallic arsenic was without therapeutic indications, physicians of the time prescribed arsenides and arsenic salts as antiperiodics, antipyretics, antiseptics, antispasmodics, caustics, cholagogues, depilatories, hematinics, sedatives, and tonics.

Early use of arsenic derivatives for infectious diseases was based on the work *Paul Ehrlich, du*ring his time, 500 types of organic arsenic compounds were in clinical use. In 1910, *Ehrlich* introduced salvarsan, an organic arsenical that could cure syphilis and is still used today to treat trypanosomiasis. Arsenic therapy subsequently became the mainstay of antileukemic treatment until its use was supplanted by radiation therapy in

the early 20th century. Inorganic arsenic was used as a therapeutic agent through the mid-twentieth century, primarily for the treatment of leukemia, psoriasis, and chronic asthma;

Table-7: Medicinal use of arsenic[102].

- Hippocrates used realgar (As_2S_2) and orpiment (As_2S_3) as remedies for ulcers.
- Dioscorides used As_2S_3 as a depilatory.
- Jean de Gorris used arsenic as a sudorific.
- Angelus Salva used arsenic against the plague.
- Lentilius and Friceius used arsenic as a treatment for malaria.
- Many preparations of arsenic were tried therapeutically in the 18th century, and several (Aiken's Tonic Pills, Andrew's Tonic, Arsenauro, Gross' Neuralgia Pills, Cholor-Phosphide of Arsenic, Sulphur Compound Lozenges) were still in circulation at the end of the 19th century.
- Arsenic was a mainstay of the 19th century Materia Medica.
- Fowler's solution (potassium arsenite) was praised for its success in treating asthma, chorea, eczema, Hodgkin's disease, pemphigus, pernicious anemia, and psoriasis.
- Ehrlich discovered an organic arsenical (salvarsan) that cured syphilis and was used to treat trypanosomiasis.
- Arsenic has been used in traditional Chinese medicine for hundreds of years, and derivatives are still used to devitalize the pulp of diseased teeth and in regimens for psoriasis, rheumatic diseases, and syphilis.

organic arsenic antibiotics were extensively used in the treatment of spirochetal and protozoal disease. The availability of inorganic arsenicals in western medicines ended in the 1970s, although they may still be encountered in non-western traditional medicines. By the 1980s, the only remaining medicinal organic arsenical was melarsoprol for treatment of the meningoencephalitic stage of African trypanosomiasis. Recently, there has been renewed interest in arsenic as a therapeutic agent, namely the use of arsenic trioxide in the treatment of acute promyelocytic leukemia (APL).

1.3.0 EXPOSURE TO ARSENIC

Exposure to arsenic can occur mainly through one or both of the two pathways, inhalation, and ingestion. Non-occupational exposure occurs primarily through the ingestion of water or/and food. Though food is more commonly the main contributor to arsenic intake but in areas where drinking waters contain relatively high levels of arsenic,

drinking water is considered to be the most important source of arsenic intake[32]. Nonetheless the situation is more complicated when the food is processed or cooked with arsenic contaminated water.

1.3.1 EXPOSURE IN GENERAL POPULATION

INHALATION[32,33, 34 37]

In the general population exposure through inhalation is negligible or almost absent except in certain situations. Urban populations have a higher exposure than rural populations. Populations living in the vicinity of coal fired power plants smelting industries and active volcanoes are likely to have considerably higher arsenic exposure through inhalation. Arsenic content of tobacco grown on soils not treated with arsenic compounds is usually between 3 mg/kg to 8 mg/kg, so tobacco use could result in some extent of arsenic exposure. Household members using coal as cooking fuel could have inhalation exposure to arsenic.

INGESTION[32 33,34 37]

Arsenic has been found in all foodstuffs, arsenic in foods is a mixture of inorganic species and organoarsenicals including arsenobetaine. The actual total arsenic concentrations in foodstuffs vary widely depending on the food type, growing conditions (type of soil, water, geochemical activity, use of arsenical pesticides) and processing techniques. The highest concentrations of total arsenic are found in seafood. Meats and cereals have higher concentrations than vegetables, fruit and dairy products. The percentage of inorganic arsenic has been estimated to be about 75% in meats, 65% in poultry, 75% in dairy products, and 65% in cereals. In fruits, and vegetables and seafood the organic species predominate, with inorganic arsenic contributing 10%, 5% and 0–10% respectively. Although ingestion of arsenic in soil and dust may not be a significant source of arsenic intake in adults, it may be significant for children, particularly in locations near industrial and hazardous waste sites. The dietary intake of total arsenic reflects in largely depends on the variability in the consumption patterns of arsenic-rich food groups (sea fish/shellfish and meats) and food items grown in arsenic contaminated soils (irrigation with contaminated water and arsenical pesticide use). Wine and mineral waters can sometimes contain several hundreds of micrograms of arsenic per litre,

probably as a result of the use of arsenic containing pesticides. Inorganic forms of arsenic have been shown to predominate in wine.

Arsenic levels in natural waters are usually low (a few µg/litre), there are several areas in the world where humans are drinking water that contain arsenic at levels higher than >100 µg/L resulting from natural geochemical activity in which case it often becomes the principal source of arsenic exposure. Exposure to arsenic may result from intake of some traditional medicine (e.g., proprietary herbal asthma medicines)

1.3.2 OCCUPATIONAL EXPOSURE[32 33 34 37]

Occupational exposure to arsenic has been observed among workers involved in mining copper, gold, lead, zinc and tin ores. Such exposure can also occur among workers involved in processing such ores. Occupational exposure may also occur among workers using or producing arsenic-containing pesticides.

Exposure is primarily through inhalation of arsenic-containing particulates, but ingestion and dermal exposure may be significant in particular situations. (e.g. preparation of CCA-treated timber). Workers may be exposed to airborne arsenic in cutting and sawing operations on wood treated with arsenic-containing preservatives. Workers in coal-fired power plants may also be exposed to arsenic found in the coal, or more likely that found in the fly ash during cleaning. There is the potential for significant occupational exposure to arsenic in industries like electronics, arsenic production, and glass manufacturing.

1.4.0 ARSENIC METABOLISM, RETENTION & EXCRETION

Absorption[32 33 37 41]

Humans are exposed to many different forms of inorganic and organic arsenic species (arsenicals) in food, water and other media. The metabolism and disposition of inorganic arsenic may depend on its valence state. The most common valence states of arsenic to which humans might be environmentally exposed are the trivalent (As^{III}) and pentavalent (As^{V}) forms and these two forms readily interconvert.

Arsenic can be absorbed from the gastrointestinal tract after ingestion of arsenic-containing food, water, beverages or medicines, or as a result of inhalation and subsequent mucociliary clearance. The bioavailability of ingested inorganic arsenic will vary depending on the matrix in which it is ingested (e.g. food, water, beverages, soil.

The solubility of the arsenical compound itself and the presence of other food constituents and nutrients in the gastrointestinal tract play an important role in the absorption of the ingested arsenic. About 95% of the ingested arsenic is absorbed. The highest absorption takes place in the small intestine; absorption from the mouth and stomach are relatively low. Soluble arsenates (As^{V}) and arsenites (As^{III}) are rapidly and extensively absorbed from the gastrointestinal tract, and arsenite is possibly more extensively absorbed from the gastrointestinal tract than arsenate at lower doses (e.g. 0.4 mg As/kg), whereas the reverse appears to occur at higher doses (e.g. 4.0 mg As/kg). Uptake of pentavalent arsenic is carried out by a saturable transport process. This absorption markedly decreases in the presence of phosphate, most likely because arsenate and phosphate can share the same transport mechanism.

Dermal absorption of arsenic could result from contact with contaminated water or soil, but this absorption is very low. About 1.9% of inorganic arsenic in solution in water and 0.8% in soil could be absorbed through the skin over a 24 hour period. Moreover inhaled arsenic is also well absorbed (40-60%).

Arsenic in blood[32,33,37,41]

Inorganic arsenic is reported to be rapidly cleared from blood. About half of the dose is accumulated in the blood, mainly in the red blood cells. The kinetics of arsenic clearance in plasma and erythrocytes are similar, although levels in erythrocytes tended to be approximately 3-fold higher a few hours after exposure. The rate of decline of arsenic in the erythrocytes was comparable with that in plasma, but the erythrocytes contained about 3 times more arsenic than the plasma 10 h after exposure. The plasma curve showed a three-compartment model. The first half-life seemed to be very short, and the bulk of the arsenic was removed from the plasma at a high rate. Some 24 hours after exposure, less than 0.1% of the dose remains. The second phase of the curve showed a half-time of about 30 hours. The third phase of the curve, beginning about 1 week after the exposure, showed a very low rate of disappearance with a half-time of over 200 hours. The predominant species of arsenic found in serum are DMA (~15–30%) and arsenobetaine (~54–76%), the remainder being protein-bound inorganic arsenic. transferrin is the main carrier protein of the inorganic arsenic. MMA are undetectable in blood.

Arsenic in Tissues[32,33,37,41,103,104]

Arsenic is widely distributed in the body both after either long-term relatively low-level exposure or poisoning. Following exposure highest levels of arsenic occur in the liver, kidney, skin, lung, intestinal mucosa and spleen. Arsenic concentrations are quite low in both blood and brain relative to other tissues and that arsenic concentration in any given tissue was quite variable. The predominant arsenical in tissues was iAs^{III}, and has been reported to account for 53 to 85% of the total tissue arsenic burden; iAs^{V} accounts for no more than 2% of the total tissue burden, while MAAs account for 10 to 32% and DMAs for 4 to 19% of the total tissue burdens. In all tissues, the concentrations of MAAs have been found to exceed those of DMAs by ratios of 2.4 to 3. In most organs, the arsenic levels fall fairly rapidly with time. In some organs, such as skin, brain, and skeleton, arsenic levels decrease more slowly. The rate of decrease of arsenic levels in the skin appear to be especially slow as high arsenic levels were still present 48 h after exposure. Quite high inter-individual variation in total tissue arsenic occur, arsenic accumulates in tissues with age and no sex-dependent differences in arsenical tissue levels have been found. In tissues inorganic arsenic has been found to be the predominant form, followed by DMA. MMA levels were uniformly low and detected only in liver and kidney. Intracellular distribution was similar after exposure to either form of arsenic in lung, liver, and kidney, where over 80% of the arsenic was found in the nuclei and cytosol.

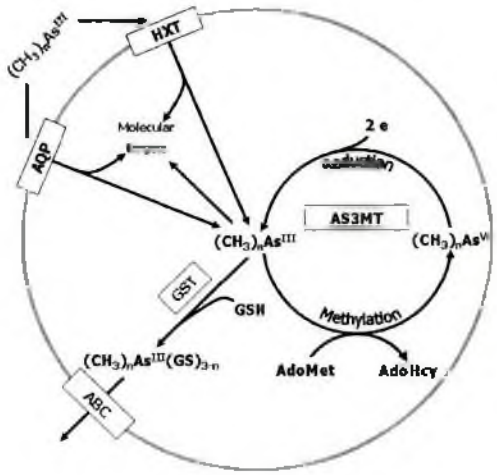
Biotransformation/Metabolic transformation[32,33,37,41,103-138]

Understanding of processes involved in the influx (cellular uptake), transformation of arsenicals in the cell (metabolism), molecular interactions between arsenicals and cellular proteins and efflux of arsenicals from cells provides a basis for understanding the modes of action by which iAs acts as a toxin. Under steady state conditions, intracellular concentrations of each arsenical species are determined by the ratio of rates of uptake and loss of that species from the cell. Rates at which intracellular concentrations of these arsenicals change are controlled by rates of efflux of metabolites.

Influx of arsenicals occurs through aquaporins (AQP), hexose permease transporters (HXT) and phosphate carrier system (figure-2 & 3). Aquaporins (AQP) are a family of membrane-spanning proteins that facilitate movement of uncharged solutes down

concentration gradients. Human AQP (hAQP7 and hAQP9) facilitates uptake of both iAs^{III} and methylarsonous acid ($CH_3As^{III}(OH)_2$, MAs^{III}). Hexose permease transporters (HXT) are another pathway for influx of iAs^{III}. Polymerization of three $As^{III}(OH)_3$ molecules may produce a substrate that resembles hexoses typically transported by HXT. These permeases could play significant roles in iAs^{III} uptake. GLUT1, a mammalian glucose permease, has been found to facilitate uptake of $As^{III}(OH)_3$ and MAs^{III}. Because AQP and HXT have different patterns of tissue distribution, it is possible that both pathways play significant roles in uptake of arsenicals into cells.

Fig No-2 : Metabolism of arsenic in a cell, incorporating molecular components involved in influx, efflux and transformation[101].

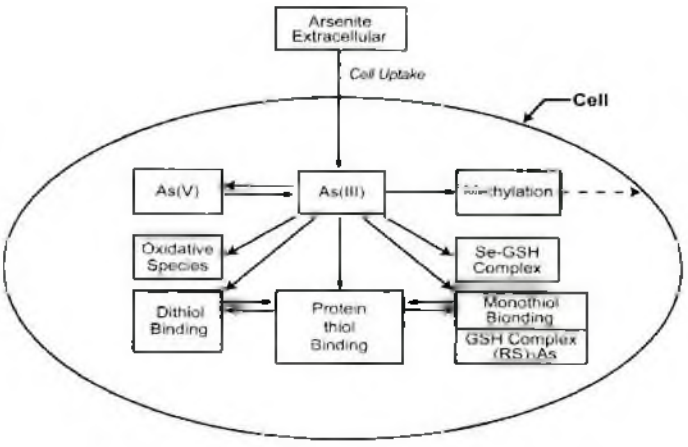


$(CH_3)_nAs^{III}$ (a trivalent arsenical species where n=0 to 3); AQP (aquaporin); HXT (hexose permease transporter); AS3MT (arsenite methyltransferase); GSH (glutathione); GST (glutathione-S-transferase); $(CH_3)_nAs^{III}(GS)_{3-n}$ (a complex of an arsenical containing n CH_3 groups (where n=0 to 3) and 3−n GSH molecules; ABC (ATP-binding cassette transporter); AdoMet, (S-adenosylmethionine); AdoHcy (S-adenosylhomocysteine).

Arsenates (As^V) enter the cell via the phosphate carrier system. Once inside the cell, it can compete with phosphate. It can bind to polyphosphates, for example, ADP, after which it is rapidly hydrolyzed (figure-4). Some of the intracellular arsenate can be reduced enzymatically to arsenite by arsenate reductase and/or monomethylarsonic acid (MMA^V) reductase (figure-4), and some of it can be excreted in the urine. The reduction of arsenate by arsenate reductase/PNP requires the presence of inosine and a thiol compound. The most active naturally occurring thiol is the vicinal dithiol dihydrolipoic acid. As lipoic acid is much more active than the monothiol GSH, arsenate and the

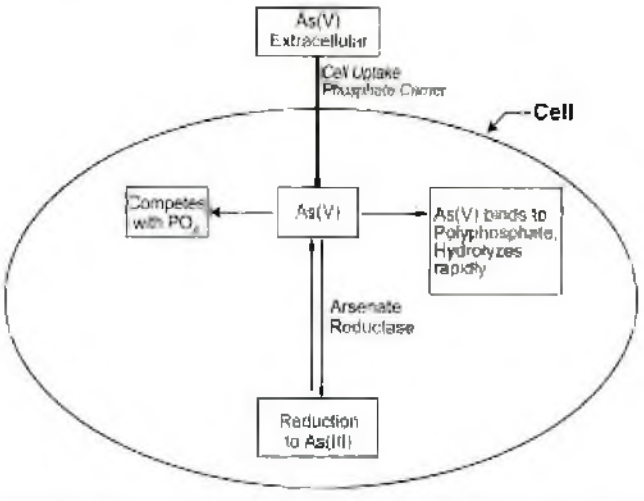
reductase appears to have a greater affinity for dihydrolipoic acid than GSH. Purine nucleoside phosphorylase (PNP) an enzyme involved in purine metabolism, has been found to have arsenate reducing activity. The reduction of arsenate can also be catalyzed by human liver MMA^V reductase/hGSTO-1.

Fig No-3 : Fate of arsenite in mammalian systems[102].



Arsenites once within the cell can be oxidized to the less reactive arsenate; it can bind to thiols such as glutathione (GSH) and thiol-containing proteins. Arsenites bind more strongly to dithiols (such as lipoic acid) and thiols that are in very close proximity to each other as part of the three dimensional structure of proteins (figure-3).

Figure No-4 : Fate of arsenate in mammalian systems[102].

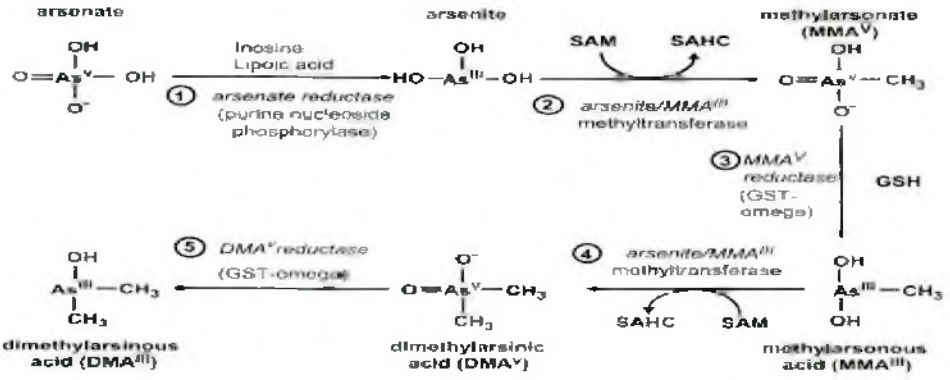


The fate of arsenic in the human body involves not only arsenate and arsenite but also monomethylarsonic acid (MMA^V), monomethylarsonous acid (MMA^{III}), dimethylarsinic acid (DMA^V), and dimethylarsinous acid (DMA^{III}). Both arsenate and arsenite are

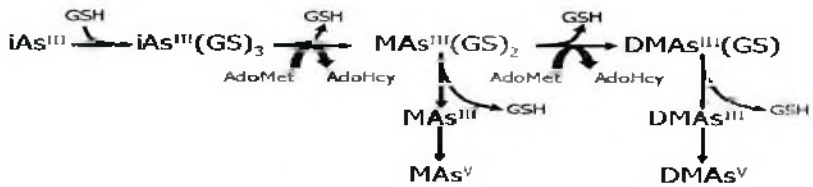
extensively methylated in humans. Biomethylation is the major pathway for the metabolism of inorganic As (iAs) in humans and other mammals. In humans, iAs is converted to methylarsenic (MAs) and dimethylarsenic (DMAs) that contain As (arsenic) in +3 (As^{III}) or +5 (As^{V}) oxidation state. Arsenic methylation activity is localized in the cytosol and appears to occur sequentially mainly in the liver, but also takes place kidney, lung and testis (male).

Three mechanisms of methylation have been proposed to exist that includes enzymatic biomethylation (figure-5a), methylation involving formation of arsenic–glutathione (GSH) complexes (figure-5b) and direct methylation involving vitamin B_{12}. In the methylation of iAs two classes of enzymes are involved including AsV-reductases and AsIII-methyltransferases.

Fig No-5: Alternative schemes for the conversion of inorganic arsenic (iAs) into methylated metabolites[101,102].



(a) Scheme for the oxidative methylation of arsenicals in which reduction of oxidized arsenicals is interposed between each methylation reaction. [SAM (S-adenosylmethionine); SAHC (S-adenosylhomocysteine); GST- omega (); GSH (glutathione)].



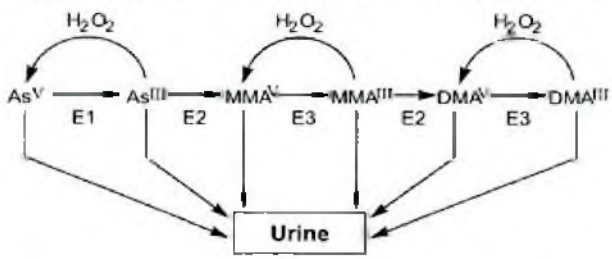
(b) Scheme for methylation of arsenic involving formation of arsenic–glutathione (GSH) complexes. [AdoMet, S-adenosylmethionine (SAM); AdoHcy, S-adenosylhomocysteine].

Arsenite is methylated by arsenite methyltransferase. and it has been proposed that the arsenic methyltransferase is a protein called CYT19. The activities for methylating arsenite and MMA^{III} are on the same protein. A thiol is required for this methylation

activity. A number of different thiols can satisfy this thiol requirement among which *S*-adenosylmethionine (SAM) has been identified as the methyl donor. In addition to methyltransferase, among other enzymes involved in the biotransformation of inorganic arsenic, the most important is MMA^V reductase. The enzyme MMA^V reductase/human glutathione-S-transferase-omega (hGSTO-1) catalyzes the reduction of MMA^V, DMA^V, and arsenate. It changes the oxidation state of the +5 metabolites to +3 resulting in MMA^{III}, DMA^{III}, and arsenite formation. MMA^V reductase is the rate-limiting enzyme for the inorganic arsenic methylation pathway. All of the +3 species are more toxic than their analogs having a +5 oxidation state. MMA^V reductase has an absolute requirement for GSH. GSTs, the enzymes involved in the detoxication of many xenobiotics or electrophiles produced from the action of cytochrome *P*450-linked oxidases have been found to have a role in arsenic methylation. Glutathione-S-transferase omega (GSTO) has been identified as catalyst for GSH-dependent reduction of MAs^V to MAs^{III}. hGSTO-1 has properties unlike those of other glutathione-*S*-transferases.

Alternatively sequential transfer of methyl groups from AdoMet (S-adenosylmethionine SAM) to an arsenical-containing substrate without oxidation of trivalent arsenic (Fig-5 b) can take place. In this non-oxidative reaction scheme, the arsenical is initially complexed by GSH to preserve the arsenic in the trivalent oxidation state. It has also been suggested that cysteine residues in cellular proteins could serve an analogous function. AS3MT is the enzyme catalyzing the methylation steps in the pathway and S-adenosylmethionine (AdoMet/SAM) is the methyl donor.

Figure No-6: The arsenate to arsenite to arsenate cycle[102,114].



E1 can be PNP or MMA(V) reductase. E2 is arsenic methyltransferase. E3 is MMA(V) reductase.

The trivalent species not only can be methylated, but once formed they also can be oxidized back to their pentavalent analogs by hydrogen peroxide produced by xanthine oxidase and ROS. In addition, the trivalent and pentavalent species are excreted to some extent in the urine of many species. ROS are formed concomitantly with the oxidation of DMA^{III} to DMA^{V} and during the potential oxidations of DMA^{III}, MMA^{III}, and arsenite, the oxidation of one might also produce hydrogen peroxide for the oxidation of others (figure-6).

Xanthine oxidase, an enzyme involved in purine metabolism produces hydrogen peroxide (H_2O_2) in cells; the biochemical reaction catalyzed by hypoxanthine–xanthine oxidase systems produces xanthine, uric acid, superoxide anion, and hydrogen peroxide. This enzyme is one of the major sources of free radicals in cells. But the xanthine oxidase reaction is not the only producer of hydrogen peroxide. The dismutation of superoxide radical via superoxide dismutase also can produce it. Other sources of hydrogen peroxide are the NADH/NADPH oxidase system, peroxisomes, aldehyde oxidase, and during L-pipecolic acid oxidation. Under basal conditions, human cells produce 2 billion superoxide anion and hydrogen peroxide molecules per cell per day. A typical human cell produces about 3 $x10^9$ hydrogen peroxide molecules per hour. Hydrogen peroxide is inactivated in a cell by catalase and peroxidases such as selenium containing glutathione peroxidase, the latter being the more important enzymes for hydrogen peroxide degradation. Other factors such as Cu, Pb, and Fe can increase the rate of oxidation of arsenite by hydrogen peroxide. Gender and ethnicity also have been found to affect hydrogen peroxide production with men producing more than women. Approximately 20 to 35% of the variation in hydrogen peroxide production can be due to genetic factors. It has also been found that arsenic can induce formation of reactive oxygen species including hydrogen peroxide.

Membrane bound efflux transporters are critical factors in removal of arsenicals from cells. These proteins use ATP hydrolysis to generate energy for transport of a wide range of substrates against concentration gradients, and are designated ATP-binding cassette (ABC) transporters. Multidrug-resistant proteins 1 and 2 (MRP1, MRP2) -members of the ABCC subfamily and multidrug-resistant/pglycoprotein (MDR/P-gp) a member of the ABCB1 subfamily have been implicated in efflux of arsenicals from cells. Decreased

influx and increased efflux of arsenic from cells results in the lower intracellular accumulation of arsenic was linked to. Increased expression of MRP1 and MDR1 has been found to be associated with cross-tolerance to acute cytotoxic effects of iAs^{III} in cells chronically exposed to methylarsonic acid ($CH_3As^{V}O(OH)_2$, MAs^{V}), or dimethylarsinic acid ($(CH_3)_2$-$As^{V}OOH$, $DMAs^{V}$) Increased expression of MRP2 and little or reduced expression of AQP3, AQP7 and AQP9 combined to limit the accumulation of arsenic in cells.

Changes in the levels and activities of various members of the GSH-S transferase (GST) family had been found to be associated with altered resistance of cells to the acute cytotoxic effects of arsenicals. GSTs are known to catalyze nucleophilic attacks of GSH on non-polar electrophiles , therefore it has been assumed that members of this enzyme family possibly catalyze formation of a bond between the thiol sulfur in the cysteine residue of GSH and iAs^{III}. Complexation of iAs^{III} by GSH to form arsenic triglutathione ($iAs^{III}(GS)_3$) has been demonstrated both in vitro and in vivo. This complexion possibly requires enzymatic catalysis by GSTP. A relatively strong and specific interaction between this protein and trivalent arsenic has been found to exist. As-GSH complexes formed enters in membrane vesicles and export of iAs^{III} by these vesicles requires GSH. GSTP1 and MRP1 have been found to be closely associated with membrane vesicles which suggest that complexation of arsenic by GSH was a prerequisite to cellular export. It was possible that cotransport of GSH and arsenic by ABC transporters could account for an apparent linkage of these processes.

The conversion of arsenite to methylated metabolites has been reported to occur at a faster rate and more extensively than the conversion of arsenate; and methylarsonous acid has been reported to be converted to DMAs more rapidly and extensively than was methylarsonic acid. The rate of biomethylation has been found to be highest in the testes but however, on a mass basis the liver is probably the major site for the methylation of arsenicals.

Excretion[32,33,37,41,103 ,105 ,139]

Inorganic arsenic is eliminated primarily via the kidneys. Arsenic is excreted in sweat to some degree. The mean concentration of arsenic in the sweat could be about 1.5 μg As/litre accounting for a calculated hourly loss of 2 μg of arsenic in a hot and humid

environment. Keratin-containing tissues-skin, hair and nails are also potential excretory routes for arsenic, although they would be quantitatively minor.

Desquamation of skin also result in the removal of arsenic calculated daily loss of arsenic through desquamation of normal skin to be is about 0.1-0.2 μg. About 0.6% of the ingested arsenic has been found to be eliminated through hair. Arsenic is also excreted in human milk at low levels. Only ~4–8% of the dose is eliminated in faeces and 66–79% is eliminated in the urine.

In the case of exposure to arsenic via drinking-water, even at very high arsenic concentrations, the methylation of arsenic seems to be relatively unaffected by the dose. MMA is also excreted in the urine of to a great extent. The proportion of MMA excreted in human urine is highly variable. Ratios of arsenic metabolites in urine (e.g. DMA/inorganic arsenic, MMA/inorganic arsenic or DMA/ MMA are often considered as indication regarding saturation or inhibition of methylation.

Dimethylarsinic acid constitutes on average 66% of the total urinary arsenic, while methylarsonic acid, iAs^{V}, and iAs^{III} accounts for 8.0, 17.0, and 8.4%, respectively. The proportion of methylated metabolites in urine can vary considerably. In occupationally and environmentally exposed population groups the average proportions of MMA and DMA in urine (range of average total urinary arsenic from 10.2 to 245 μg/L) has been found to range from 9 to 20% and 61 to 70%, respectively.

Inorganic arsenic metabolism is known to be affected by liver disease in humans. Liver disease had no effect on the total amount of arsenic excreted within 24 h, but dramatically shifted the proportion of MMA and DMA excreted in the urine. The percentage of arsenic excreted as MMA has been found to decrease in liver disease patients and the percentage of DMA was found to increase. In patients with other non-hepatic disease, the arsenic methylation was found not to be affected.

Recently MAA^{III} and DMA^{III} has identified in urines from individuals from two different geographical regions who were chronically exposed to arsenic from drinking water and were not treated with chelators. Some of the MAA^{III} and DMA^{III} excreted in urine by individuals chronically exposed to iAs could originate in the liver, be released into the systemic circulation, be cleared by the kidney and accumulated in the contents of the urinary bladder, and excreted in urine. If this pathway for formation and clearance of the

methylated arsenicals that contain As^{III} was correct, these species are could be reasonably stable under physiological conditions.

Retention and turnover[32,33,37,41,103,105,139]

Quite high inter individual variation for retention exists. Retention was higher for arsenite than for arsenate. Both MAAs and DMAs are also retained in tissues. Much of the arsenic in tissues are arsenite (iAs and methylated arsenicals) has been shown to be protein bound. Proteins that bind arsenicals include constitutively expressed proteins and proteins expressed by the presence of arsenicals in cells. Soluble proteins, that binds arsenicals range from about 37 kDa to >2000kDa. Lungs too, tenaciously retain DMAs .

Differences among tissues in the retention of iAs, MAAs, and DMAs has been reported. In blood, kidney, and lung, DMAs was the predominant arsenical beyond four hours post dosing; only in liver iAs has been found to remain the predominant arsenical at all time points. Differences among tissues in the rate of formation of methylated arsenical species and also differences in the translocation of arsenicals among tissues have been reported. The retention of arsenicals in tissues may be influenced by the presence of specific proteins that bind arsenicals. Specific proteins are thought to mediate the control of the distribution and retention of iAs, MAAs, and DMAs. Exposure to iAs^{III} has been found to increase at least 4 proteins (19.5, 38.5, 42 and 59kDa). MAAs was predominantly bound to the 130 kDa protein fraction and DMAs was predominantly bound to the 37 kDa protein fraction. Cells have been found to express Galectin 1 (Gal 1) and in such cells increased accumulation of arsenic in the form of Gal 1-iAs^{III} complex has been reported.

Of the ingested arsenate about 65.9% of the dose is cleared with a half-life of about 2.09 days, 30.4% with a half-life of about 9.5 days and 3.7% with a half-life of about 38.4 days. Such information relating to trivalent inorganic arsenic is lacking.

Reaction with body components[32,33,37,41, 103,105,139]

Basic differences in the interaction of pentavalent and trivalent inorganic arsenic with body components that exists, is an important determinant in observed differences in tissue distribution. As iAs^{V} can act as a phosphate analogue at the molecular level this means that arsenate can compete with phosphate for active transport processes. This could be important as addition of phosphate can decrease intestinal uptake and renal tubular reabsorption of arsenate. Arsenate can also substitute for phosphate in the

hydroxyapatite crystal of bone, leading to accumulation of arsenic in bones. At the biochemical level, arsenate can substitute inorganic phosphate in the synthesis of ATP thus uncouple oxidative phosphorylation in mitochondria; it can also inhibit glycolysis by competing with phosphate to form the dysfunctional compound 1-arseno-3-phosphoglycerate, rather than 1:3-diphosphoglycerate.

Arsenite reacts readily with vicinal sulfhydryl groups of a variety of essential enzymes and proteins. It is the affinity of arsenite for sulfhydryl groups that accounts for its accumulation in keratin-rich tissues such skin, hair and nails. Arsenite also interacts with the ubiquitous sulfhydryl-containing cellular tripeptide GSH at many different levels in the methylation process.

1.5.0 MECHANISM OF ARSENIC TOXICITY AND FACTORS MODIFYING TOXICITY

MECHANISM OF ARSENIC TOXICITY[32,33,37,41,103,105,139]

As iAs is readily converted into MAAs and DMAs (including MAs^{III} and $DMAs^{III}$), it is relevant to consider the biological effects of methylated arsenicals. Trivalent arsenicals have been found to readily react with sulfhydryl groups (such as GSH and cysteine), the complex between arsenic and vicinal sulfhydryl is particularly strong. The activity of enzymes or receptors is due in part to the functional groups on amino acids such as the sulfhydryl group on cysteine, or coenzymes (such as lipoic acid) which has vicinal thiol groups. Thus, if arsenite binds to a critical thiol or dithiol the enzyme may be inhibited. Trivalent arsenicals have been reported to have the ability to inhibit more than 100 enzymes. Arsenicals are genotoxins, both MAA^{III} and DMA^{III} were found to be manyfold more potent inducers of DNA damage than was arsenite. Exposure to arsenite, arsenic trioxide, or arsenate, results in the generation of reactive oxygen species (ROS). ROS produced by the metabolism of DMA^{III} have been implicated in the induction of single-strand (ss) breaks in DNA from the lungs MAA^{III} induces the release of iron from ferritin, liberated iron induces ROS formation, and ROS causes nicking of DNA.

Trivalent inorganic arsenicals (arsenites), inhibits pyruvate dehydrogenase a lipoic-acid-dependent enzyme involved in gluconeogenesis. Inhibition of gluconeogenesis could ultimately result in depletion of carbohydrates. Arsenite inhibits the binding of steroids to

the glucocorticoid receptor. The glucocorticoid receptor has vicinal thiols that are involved with steroid binding. Arsenite is more toxic than arsenate, as evidenced by the lower amount of it needed to elicit a toxic response.

ROS generation by cells exposed to iAs^{III} species has been linked to the activation of NF-kB, a transcription factor which is activated by oxidative stress and to the stimulation of NF-kB-dependent gene transcription. The induction of oxidative stress resulting in modification of redox sensitive signaling molecules, including NO, *S*-nitrosothiols, AP-1, NF-kB, IkB, p53, and p21ras, affects gene transcription and is likely an important mechanism involved in the induction of cellular injury by As.

Trivalent organic arsenicals (MMA^{III} and DMA^{III}) also react with sulfhydryl groups, as trivalent inorganic arsenicals. MMA^{III} has been found to be the most cytotoxic species among the trivalent arsenicals. Binding of MMA^{III} and DMA^{III} to protein occurs to a greater extent than the pentavalent organic forms (MMA^{V} and DMA^{V}). This arsenic (MMA^{III} and DMA^{III}) and cellular protein interaction is possibly the basis of the toxic effects of arsenic exposure. MAs^{III} and $DMAs^{III}$ have been found to be significantly more toxic to hepatocytes, epidermal keratinocytes, bronchial epithelial cells and bladder epithelial cells than arsenate, arsenite, MAs^{V} and $DMAs^{V}$. Methylated trivalent arsenicals are potent inhibitors of GSH reductase. The activity of these chemicals is greater than inorganic trivalent arsenic and the pentavalent organic arsenicals. GSH reductase contains five cysteine residues in each dimeric unit, which may provide a binding site for trivalent arsenic to inactivate the enzyme. Inhibition of GSH reductase could result in GSH depletion.

Complexes of arsenite or MAA^{III} with either L-cysteine or GSH were more potent inhibitors than were the parent arsenicals. The most potent inhibitor in this series of compounds was MAs^{III}-diglutathione ($MAs^{III}(GS)_2$). Arsenothiols and arsenicals containing As^{III} are potent inhibitors of thoredoxin reductase (TR). Because cells rapidly convert arsenite to MAs and DMAs, the progressive loss of TR activity was possibly related to the accumulation of the metabolites, MAs and DMAs, in cells rather than the accumulation or persistence of iAs in cells.

Pentavalent inorganic arsenicals (arsenates) replace phosphates. Arsenate can form esters with glucose and gluconate forming glucose-6-arsenate and 6-arsenogluconate,

respectively. These compounds resemble glucose-6-phosphate and 6-phosphogluconate. Glucose--6-phosphate and glucose-6-arsenate have similar *K*m and *V*max values as substrates for glucose-6-phosphate dehydrogenase and each can inhibit hexokinase. Arsenate uncouples oxidative phosphorylation because it has a similar structure to phosphate. Two mechanisms for this effect, termed arsenolysis, have been proposed. During glycolysis, arsenate can substitute for phosphate to form 1-arsenato-3-phospho-D-glycerate, instead of 1,3-biphospho-D-glycerate, from D-glyceraldehyde-3-phosphate. The arsenic anhydride is unstable and hydrolyses to arsenate and 3-phosphoglycerate. Normally adenosine-5′-triphosphate (ATP) is generated in this reaction, but with arsenate present instead of phosphate, ATP is not formed. Adenosine-5′-diphosphate-arsenate is synthesized by submitochondrial particles from adenosine-5′-diphosphate (ADP) and arsenate in the presence of succinate. ADP-arsenate hydrolyses more easily than ATP, the formation and hydrolysis of ADP-arsenate results in arsenolysis.

Pentavalent organic arsenicals (MMA^V and DMA^V) are reduced by thiols to trivalent organic arsenicals which then bind other thiols. The reduction of organic pentavalent arsenicals to their trivalent forms, as observed with inorganic pentavalent arsenicals, is a potential mechanism of action of the pentavalent organic arsenicals.

It has been suggested that dimethylarsine ($(CH_3)_2As^{III}H$), a product of the reduction of DMA^V, with molecular oxygen, yields a peroxyl radical, $(CH_3)_2AsOO^{\bullet}$, and a superoxide anion radical (O_2^{-}). And this peroxyl radical could possibly play a major role in the interactions with DNA and produce ss breaks in DNA,

In vitro studies have revealed that arsenic could have the following biochemical effects:

1. Induction of oxidative damage to DNA.
2. Altered DNA methylation and gene expression.
3. Changes in intracellular levels of mdm2 protein and p53 protein.
4. Inhibition of thioredoxin reductase (MMA^{III}, but not As^{III}).
5. Inhibition of pyruvate dehydrogenase.
6. Altered colony-forming efficiency.
7. Formation of protein-DNA cross-links.
8. Induction of apoptosis.

9. Altered regulation of DNA repair genes, thioredoxin, glutathione reductase, and other stress-response pathways.
10. Stimulation (at very low doses) and inhibition (at higher doses) of normal human keratinocyte cell proliferation.
11. Altered function of the glucocorticoid receptor.

FACTORS MODIFYING TOXICITY[32 33,37,41,139,140]

a) Genetic Polymorphisms Related to Arsenic Metabolism

Glutathione *S*-transferases (GST) constitute a large family of detoxifying enzymes that catalyze the conjugation of reduced GSH with a wide range of compounds. Glutathione (GSH) are essential for the reduction and methylation of arsenic, and as GSTs influences GSH levels, GSTs are as possible candidates for influencing the metabolism and the toxicity of arsenic. Polymorphisms in that GST also might affect both cellular protective mechanisms and arsenic metabolism. Variations observed in the pattern of arsenic metabolites in urine are an indication of a possible genetic polymorphism in the regulation of enzymes responsible for arsenic methylation. In particular, the reduction of MMA^{V} to MMA^{III} and subsequent methylation of MMA^{III} to DMA^{III} might be slow in certain people, resulting in high MMA concentrations in urine. The high urinary MMA concentrations might also be the result of low binding affinity of MMA to a carrier protein or more efficient mechanisms for excreting MMA from the cell.

b) Inter-individual Variation in Arsenic Metabolism

In populations highly exposed to arsenic in drinking-water findings indicate that methylation patterns are not highly correlated with exposure level, and that a high level of interindividual variability exists. Factors such as smoking, gender, age, years of residence and ethnicity could possibly account for only ~20% of the variation observed.

Children could possibly be more sensitive to arsenic than adults. Children exposed to arsenic have been found to have a significantly higher percentage of inorganic arsenic and a lower percentage of DMA in the urine, compared with adults. An increase in the proportion of DMA in urine with an increase in age might occur in adults

Gender differences in arsenic metabolism generally do not exist, but pregnant women have been found to excrete relatively more DMA in urine than men. Pregnant women in the third trimester were reported to have more than 90 % DMA in plasma and urine, a

percentage that was significantly higher than that in non pregnant women. Low dietary intake of protein, choline and methionine has found to result in decreased excretion of DMA. It is speculated that much of the observed inter-individual variation might be explained by genetic differences in the activity of methylating enzymes and related co-factors.

c) Effect of Dose on Arsenic Methylation

The average distribution of arsenic metabolites in urine following exposure to high doses that are in the range of found in drinking water been reported as 1-6% iAs^{V}, 11-14% iAs^{III}, 10-20% MMA, and 63-70% DMA. A significant decrease in the percentage of DMA and a concurrent increase in the percentage of inorganic arsenic and MMA in urine with increasing exposure to arsenic had been reported. The effect of arsenic dose on the methylation efficiency seems to be small and mainly affects the ratio of MMA to DMA. The formation of reactive As^{III} intermediates cannot be excluded. Furthermore, the increased excretion of MMA at higher doses can indicate that the second methylation step is inhibited by iAs^{III} in the tissues. It is probable that the decrease in methylation with increasing dose was due to inhibition of methyltransferase. Methylation of arsenic, especially the second methylation step in which MMA is converted to DMA, is inhibited by excess amounts of arsenic

d) Thiols[32,33,37,41,103,139]

GSH non-enzymatically reduces arsenate to arsenite. The reduction requires 2 moles of GSH to 1 mole of arsenate and also results in the formation of oxidized GSH (GSSG). Arsenite then reacts with GSH to form a GSH–arsenite complex. The complex consists of 3 moles GSH per 1 mole arsenite and is termed arsenotriglutathione. The complex is stable over a pH range of 1.5–7.5. Arsenite has a higher affinity for a vicinal thiols, such as that in DMSA (disodium methylarsonate), than a dithiol located in GSH. Arsenite can also complex with GSH within intact erythrocytes. GSH appears to have a role in protecting the kidney from arsenite-induced toxicity. The hepatotoxicity is characterized by an increase in activities of plasma enzymes (glutamic pyruvic transaminase and glutamic oxaloacetic transaminase), which return to normal levels 48 h after exposure to arsenite.

The pentavalent organic arsenicals MMA and DMA are reduced by thiols such as GSH to the trivalent form which can then complex with the thiols. During the reduction, oxidized thiol such as GSSG is formed. MMA and DMA have been found to be bound to GSH. Because of the reactivity of trivalent arsenicals with sulfhydryl groups, the possibility exists that MMA and DMA were reduced to trivalent forms *in vivo* and then reacted with sulfhydryl groups on the proteins. The trivalent organoarsenicals and complexes are at least 10-fold more potent inhibitors than are arsenite and arsenate. The methylation of inorganic arsenic may yield metabolites that alter the cellular oxidation status by potently inhibiting the reduction of glutathione disulfide. The alteration of the oxidation status of the cell by these arsenicals may lead to more serious cytotoxic effects. Depletion of intracellular GSH has been reported to enhance cytotoxicities of arsenite, arsenate, iAs^{V} MAs^{III} but decreases the cytotoxicity of $DMAs^{V}$. $DMAs^{V}$ has been found to induce cellular apoptosis in presence of normal levels of intracellular GSH; it is possible that $DMAs^{V}$ is first reduced to $DMAs^{III}$ by intracellular GSH which ($DMAs^{III}$) in turn produces cell apoptosis.

e) Effect of Other Chemicals on Arsenic Methylation

Arsenic methylation might also be influenced by simultaneous exposure to other exposures minerals. The methylation of inorganic arsenic to MMA was found to be inhibited by vanadium, iron, selenite, and cadmium. Specifically, selenite, mercury, lead, and chromium inhibit the second methylation step. Chelating agents, such as DMPS, has been found to change the urinary arsenic metabolite pattern dramatically. In particular, urinary excretion of MMA increased. Of total arsenic in urine, the percentage of MMA increased by about 10-fold and the percentage of DMA decreased following treatment with DMPS. Citrate had a limited stimulatory effect on both steps in the arsenic methylation.

f) Effect of nutritional factors on Arsenic Methylation

In the case of arsenic, interaction or modification of effects by poor nutrition could potentially increase susceptibility. Poor nutrition might indicate an increased susceptibility to arsenic toxicity, leading to reduced methylation of arsenic and therefore increased tissue retention of arsenic. Intake of diets low in amounts of methionine, choline, or proteins could result in a marked decrease in the urinary excretion of DMA

and increased tissue retention of arsenic. Deficiency in folate and vitamin B_{12} might lead to decreased levels of S-adenosylmethyltransferase (SAM), increased levels of serum homocysteine, and possibly hypomethylation. Thus, deficiency in those vitamins might result in decreased methylation of arsenic. A synergistic interaction was seen between duration of arsenic exposure (through drinking water) and low serum β-carotene concentrations in the development of ischemic heart disease. Selenium status has also been suggested as an influence on the toxicity of arsenic. Excess intake of selenium might result in a decreased methylation of inorganic arsenic. The second methylation step, MMA to DMA, seemed to be more sensitive to the inhibitory effect of selenium as the ratio of DMA to MMA decreased significantly with selenium treatment.

g) Effect of liver diseases on arsenic methylation

The methylation of arsenic appears to be affected by hepatic disease. In various liver diseases (i.e., alcoholic cirrhosis, chronic hepatitis, homochromatosis, post-necrotic cirrhosis, steatosis, and biliary cirrhosis) decreased the proportion of MMA (in relationship to the total urinary excretion of metabolites of inorganic arsenic) and increased the proportion of DMA

1.6.0 HEALTH EFFECTS OF ARSENIC[32,33,37-41,139,141-149]

Chronic exposure to arsenic has long since been linked to adverse health effects in human populations. Arsenic is a known carcinogen and has potentialities of producing cancers at multiple sites, notably in the skin, bladder, kidneys, prostate and lungs[32,33,37-41,139,141,142,143]. Arsenic is also known to have many non-cancer health effects[32,33,37-44,139,141,143] that includes: pigmentary changes of the skin (melanosis and leucomelanosis) and keratosis of palms and soles; respiratory illnesses; hypertension; diabetes mellitus; neurological effects; adverse reproductive effects (increased fetal, natal and neonatal mortalities; elevations in low birth weight, spontaneous abortions, preterm births, stillbirths and pre-eclamsias; and congenital malformations); cardiovascular and cerebrovascular diseases; and peripheral vascular disease.

1.7.0 ALLOWABLE CONCENTRATIONS OF ARSENIC IN DRINKING WATER [141,142, 148,151]

Based on the increasing awareness of the toxicity of arsenic, particularly its carcinogenicity, and on the ability to measure it quantitatively, W.H.O had recommends guideline values for arsenic in drinking water. These guideline values are periodically reconsidered in light of available scientific information. The 1958 W.H.O International Standards for Drinking-water recommended a maximum allowable concentration of 0.2 mg/L for arsenic, based on health concerns. In the 1963 International Standards, this value was lowered to 0.05 mg/L, which was retained as a tentative upper concentration limit in the 1971 International Standards. The guideline value of 0.05 mg/L was also retained in the first edition of the Guidelines for Drinking-water Quality, published in 1984. A provisional guideline value for arsenic was set at the practical quantification limit of 0.01 mg/L in the 1993 Guidelines, based on concern regarding its carcinogenicity in humans.

Table-8: Currently Accepted National Standards of Selected Countries for Arsenic in Drinking Water[142]

Country/region	Standard: µg/ L	Country/region	Standard: µg/ L
Australia (1997)	7	Cambodia	50
European Union (1998)	10	China	50
Japan (1993)	10	India	50
USA (2002)	49	Lao PDR (1999)	50
Vietnam	10	Myanmar	50
Canada	25	Nepal	50
Bangladesh (1997)	50	Pakistan	50

1.8.0 GROUNDWATER ARSENIC CONTAMINATION SITUATION GLOBAL SENARIO [32-36,138,140,142]

Drinking water, depending on local availability is derived from surface water- ponds, rivers, lakes and reservoirs; groundwater (aquifer) and rainwater. In various parts of the world, aquifers (groundwater) having arsenic at concentrations above 50 μgmL^{-1} have been identified. Such occurrences have been reported from parts of Argentina, Austria,

Fig. 7: Distribution of documented arsenic problems in groundwater major aquifers as well as water and environmental problems related to mining and geothermal sources. Areas in blue are lakes[84].

Bangladesh, Brazil, Chile, China, Cambodia, Cambodia, France, Ghana, Greece, Hungary, Iceland, India (Madhya Pradesh and West Bengal), Inner Mongolia, Nepal, Japan, Mexico, Myanmar, New Zealand, Romania, South Africa, Taiwan, Thailand, Nepal, Vietnam, many parts of the USA (particularly the SW), and Zimbabwe (figure-7). These include natural sources of enrichment as well as mining-related sources. Mining related arsenic problems in water have been identified in many parts of the world, including Ghana, Greece, Thailand and the USA. Arsenic associated with geothermal waters has also been reported in several areas, including hot springs from parts of Argentina, Japan, New Zealand, Chile, Kamchatka, Iceland, France, Dominica and the USA[32-36,84,142].

In Asia[74,77,83,84,142,146,151-154] before 2000, arsenic contamination in groundwater was only known in Bangladesh, West Bengal of India, and sites in China. But between 2000 and 2005, arsenic-related groundwater problems have emerged in different Asian countries, including new sites in China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan, DPR Korea, and Pakistan. There are reports of arsenic contamination from Kurdistan province of Western Iran and Viet Nam (figure-8). In India besides West Bengal, arsenic contamination of ground water has been found in Arunachal Pradesh, Andhra Pradesh, Assam, Bihar, Jarkhand, Manipur, Nagaland, Tripura and Uttar Pradesh[142,146,154,155].

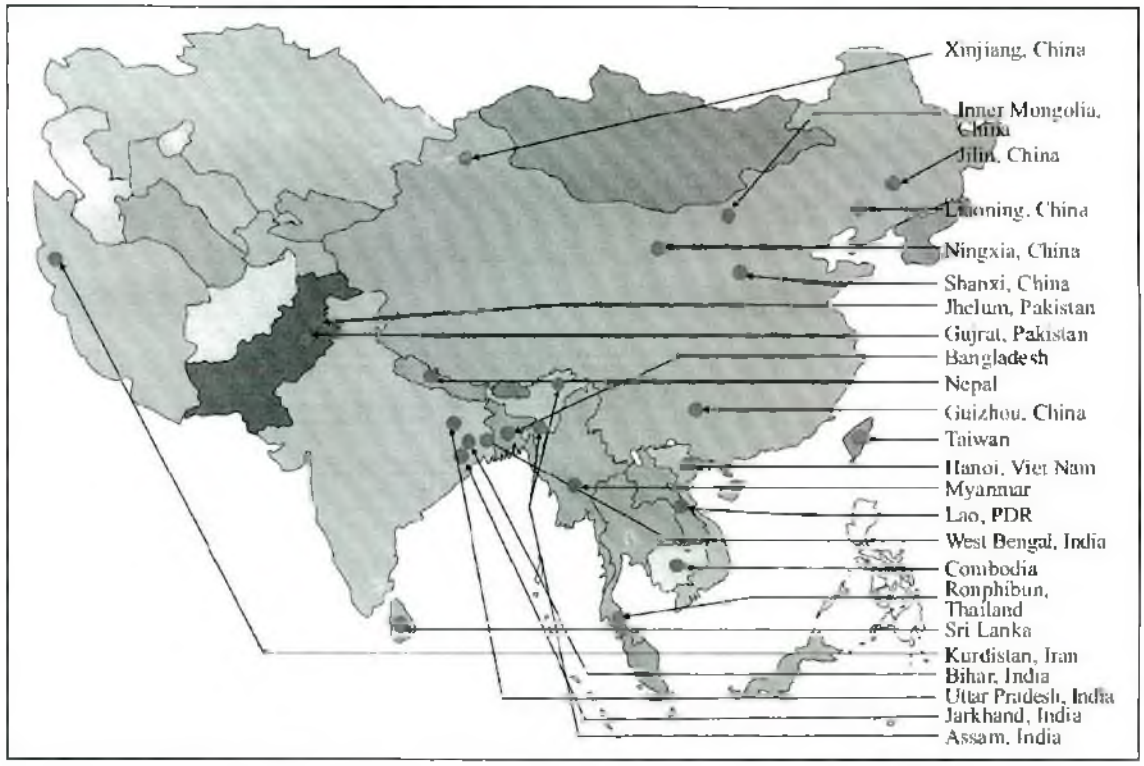


Fig 8: Distribution of Asian countries with documented arsenic problems in groundwater aquifers[146].

The southwest coastal zone of Taiwan was perhaps the first area to be identified as a problem area for health effects arising from chronic arsenic exposure. The Chaco-Pampeon Plain of central Argentina perhaps is the largest region of high-arsenic groundwaters known, covering around one million km^2. In terms of population exposed,

Table No-9: Naturally occurring arsenic problems in ground waters around the world[77,83,142,146,151,152]

Country/Region	Population Exposed	Area (km^2)	Maximum concentration (ppb)
Bangladesh	30-35 million	150000	25000
India (West Bengal)	6 million	23000	3200
China (Inner Mongolia, Xinjiang, Shanxi)	5.6 million	68000	4400
Argentina	2 million	1000000	5300
Nepal	46-75 thousand	30000	600
Chile	0.5 million	125000	1000
Mexico	0.4 million	32000	620, 1070
USA (south west)	0.35 million	206300	2600
Taiwan	0.1 million	6000	1800
Mongolia (Huhhot Basin)	0.1 million	4300	2400
Vietnam	10 million	1000	1800

arsenic problems in ground water from the alluvial deltaic aquifers of Bangladesh and West Bengal represent the most serious occurrences identified globally.

1.9.0 ARSENIC CONTAMINATION BANGLADESH SENARIO

1.9.1 ARSENIC IN DIFFERENT ENVIRONMENTAL MEDIA OF BANGLADESH

Among the different environmental media information relating arsenic in the soil, rivers, and ground water of Bangladesh are some what available. But information on arsenic concentration(s) in air of Bangladesh could not be retrieved.

Soil[71,156,157]

Significantly high arsenic concentrations have been found in agricultural soil irrigated with arsenic contaminated groundwater. In Bangladesh the average concentration of arsenic in alluvial sand and mud/clay has been reported to be 2.9mg/kg and 6.5mg/kg respectively. A concentration varying from 1.5 to 19 mg/kg has been found in Samta village of Jessore of which higher concentrations were found in the top layers of the soil. Concentrations as high as 51mg/kg and 83 mg/kg have been reported in soils of Faridpur and Comilla districts respectively.

River[55, 158]

Arsenic concentrations in seven river water samples from Bangladesh have been reported in the range <0.5–2.7 μg/L but with one sample having a high concentration of 29 μg/L. The arsenic content of surface water of these rivers is extremely low and ranges from negligible to 2.25 ppb. Arsenic content of sediment carried by the Jamuna-Padma (Ganga)-Meghna river system has been found to vary with particle size: 1.002 to 2.983 mg/kg in sand, 1.858 to 3.943 mg/kg in silt and 3.525 to 6.476 mg/kg in clay. The average arsenic content in the sediment from the River Ganges was found to be about 2.0 mg/kg (1.2–2.6 mg/kg), that from the Brahmaputra River was found to be about 2.8 mg/kg (1.4–5.9 mg/kg) and that from the Meghna River was found to be about 3.5 mg/kg (1.3–5.6 mg/kg). The total arsenic in the sediments of these rivers ranges from 1.021-3.525 mg/kg in the pre flood period and from 4.067-5.466 mg/kg in the post flood period

indicating the transport of arsenic from upstream. Higher levels were detected in clay (3.52-6.48 mg/kg) than in silt (1.86-3.94 mg/kg) or sand (1.00-2.98 mg/kg).

Groundwater[55,159,160,161,162,163]

Deep wells, tapping depths greater than 150–200 m, almost invariably have low arsenic concentrations, less than 5 μg/L and usually less than 0.5 μg/L. Wells from the older Plio-Pleistocene sediments of the Barind and Madhupur Tracts have low arsenic concentrations. The worst-affected areas of Bangladesh are to the south and east of Dhaka where in some villages, more than 90% of the wells have arsenic concentrations above 50μg/L. The ground waters are predominantly reducing, as evidenced by the major-element chemistry described above. Arsenic speciation studies have revealed a large range in the relative proportions of dissolved arsenate and arsenite. The modal proportion of arsenite appears to be between 50% and 60% of the total arsenic. Reducing arsenic-rich groundwaters from Bangladesh have As^{III}/AsT ratios varying between 0.1–0.9 but are typically around 0.5–0.6.

1.9.2 GROUNDWATER AQUIFER SYSTEM IN BANGLADESH[55, 141,159, 164]

Groundwater occurs at very shallow depths all over the country where the major aquifers are the Holocene alluviums and fan deposits and Pliocene fluvio-deltaic (Dupi Tila) sediments. Mio-Pliocene Tipam sands form minor aquifers in the hilly areas. The aquifers are highly transmissive and generally multi-layered. The aquifer conditions vary from unconfined to leaky-confined in the shallow alluvial deposits and confined in the Dupi Tila and deeper alluvial deposits. In the delta area the thickness of the recent sediment is higher and the Dupi Tila sandstone lies at greater depths. In the southern part (often called coastal plain) the thickness of the alluvial deposit is highest compared to other parts of the country. The aquifer system in Bangladesh, are geologically controlled and depends on the sedimentary characteristics, depositional environments, tectonic features and other related parameters. From geological point of view the aquifers are grouped as:

1) Pilo- Pleistocene aquifers (deep aquifer)
2) Late Pleistocene-Holocene aquifers
    i) Upper Holocene aquifer
    ii) Middle Holocene aquifer

iii) Lower (early) Holocene aquifer

The Dupi Tila sandstone constitutes the Pilo- Pleistocene aquifers. In the Pleistocene uplands that includes the Madhpur and Barind Tract the Pilo-Pleistocene aquifers lies 150 to 460m beneath a thick silty clay layer of Pleistocene age. Beneath the Dupi Tila, from above downwards lies Girujan Clay, Tipam Sandstone, Bokabil, Bhuban and other formations at greater depths. The sandstones of these formations contain water and are considered as good aquifers. The thickness and depth of the deep aquifer depends on the geological nature of the area. The deep aquifer generally lies about 200 m below the ground level. It lies at depths of 150-460 m in the red soil area, 210-300 m in costal areas and 30m at foot hills.

The shallow aquifers (alluvial and Dupi Tila sands within a depth of 200 m) and deeper aquifers (occurring at depths greater than 200 m) are separated by a thick blanket of clay. The shallow aquifers are further subdivided into upper and lower parts.

Late Pleistocene-Holocene aquifers: In the Ganges-Brahmaputra-Meghna flood pain which covers 40% of Bangladesh the Pleistocene Clay is covered by Holocene alluvial deposits of variable thickness. The aquifers lie mainly in the Holocene sediments. These Holocene aquifers are divided into 3 major divisions. Each of these divisions contains a number of interconnected sand layers. Horizontally these aquifers extend in all directions only for short distances.

Late Pleistocene-Early Holocene aquifers (Lower Holocene aquifers) are characterized by the gravel bed and sand with scattered gravels. The gravel bed has an average thickness of 10 meters and a gradient of 3/10000 towards south. The depth of the gravel bed varies (generally within 18 to 100m) from place to place. This aquifer contains huge amount of water.

Middle Holocene aquifers which overlie the Late Pleistocene-Early Holocene Aquifers are composed mainly of fine sand and have coarser sand in the upper part. The upper part contains silt and peaty organic matter. The sandy sequence varies greatly both vertically and horizontally. In the floodplain and deltaic areas of Bangladesh these mid-Holocene aquifers are often considered as the main or lower shallow aquifers. These are the aquifers mainly tapped by most hand tubewells as water source.

Upper Holocene aquifer also known as upper composite aquifer or upper shallow aquifers has two parts. The lower part is composed of silt and clay at the bottom, and fine sand at the top. While the upper part is composed of silt and clay commonly mixed with medium sand. Upper Holocene aquifer is not universal in all floodplain and deltaic areas.

The age of the groundwater at 10– 40 m depth in western Bangladesh is in the order of decades. Shallow groundwaters from south-central Bangladesh are also modern; the groundwater from 150 m was notably older with a model age of about 2000 years. Deep groundwaters from southern Bangladesh have been found to be even older with an age of 2,000–12,000 years. Deep groundwaters in southern Bangladesh were found to be even older with an age of 2,000–12,000 years.

The reasons for the distinction between groundwater arsenic concentrations in the shallow and deep aquifers of the Bengal Basin are not yet well-understood. Differences between the sediments at depth may occur in terms of absolute arsenic concentrations and in the oxidation states and binding properties of the arsenic to the sediments. However, it is also possible that the history of groundwater movement and aquifer flushing in the Bengal Basin has been important in generating the differences in dissolved arsenic concentrations between the shallow and deep aquifers. Older, deeper sediments have been subject to longer periods of groundwater flow, aided by greater hydraulic heads during the Pleistocene period when glacial sea levels around the Bangladesh landmass were up to 130 m lower than today. Flushing of the deeper older aquifers with groundwater is therefore likely to have been much more extensive than in the Holocene sediments deposited during the last 5000–10000 years. Hence, much of the arsenic in the deep sediments may have previously been flushed away.

1.9.3 SOURCE OF THE ARSENIC IN BENGAL DELTA AND POSSIBLE MECHANISMS OF ARSENIC RELEASE FROM AQUIFER SEDIMENTS

GENESIS OF BENGAL BASIN [165-174]

The Bengal basin stands on what once was part of the Tethys Ocean ~55 Ma years ago, before the India approached and ultimately collided with Asia. The Bengal basin is one of the largest sedimentary basins of the world. Bangladesh occupies most of the present day

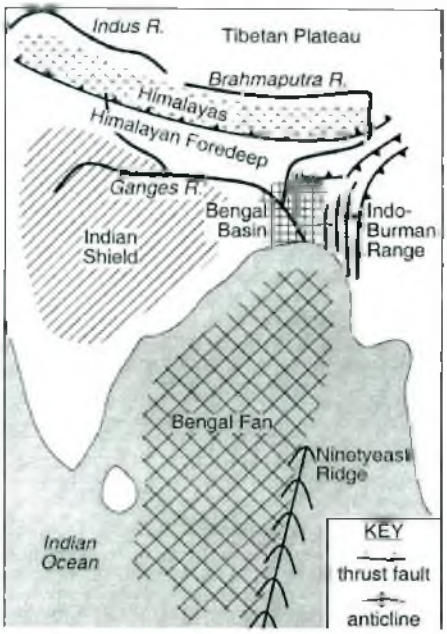


Fig No-9: Tectono-sedimentary map of the Indo-Asian collision showing the Bengal Basin that receives sediments carried by Ganges & Brahmaputra rivers and it's location along a tectonically active trailing-edge margin surrounded by the Indian craton, Himalayan foredeep & Indo-Burman fold belt[166,167].

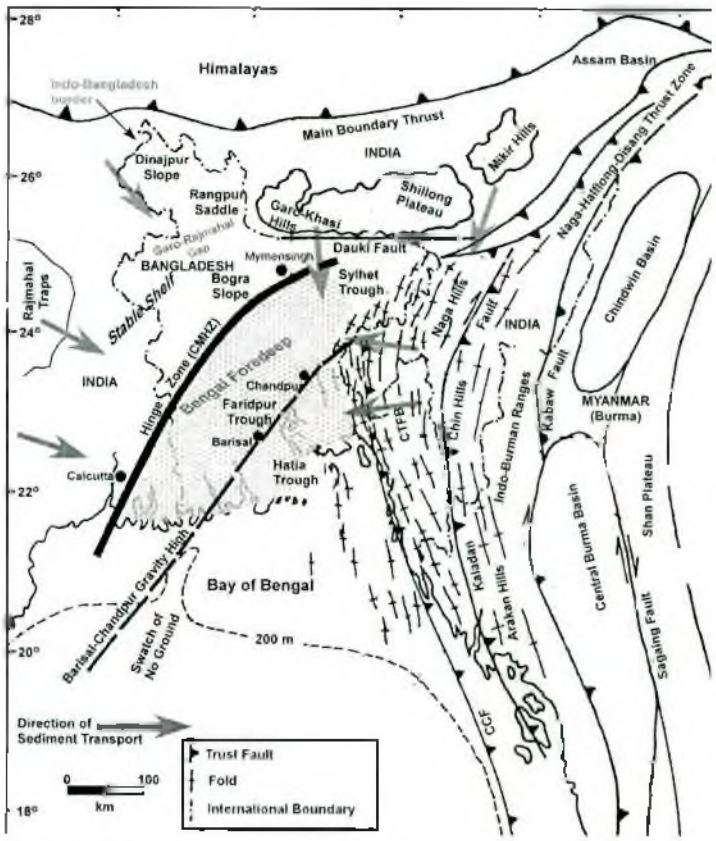


Fig No-10: Map of the Bengal basin and surrounding areas, showing the directions of sediment transport[169].
CCF: Chittagong-Cox Bazar fault; CTFB: Chittagong-Tripura fold belt[c].

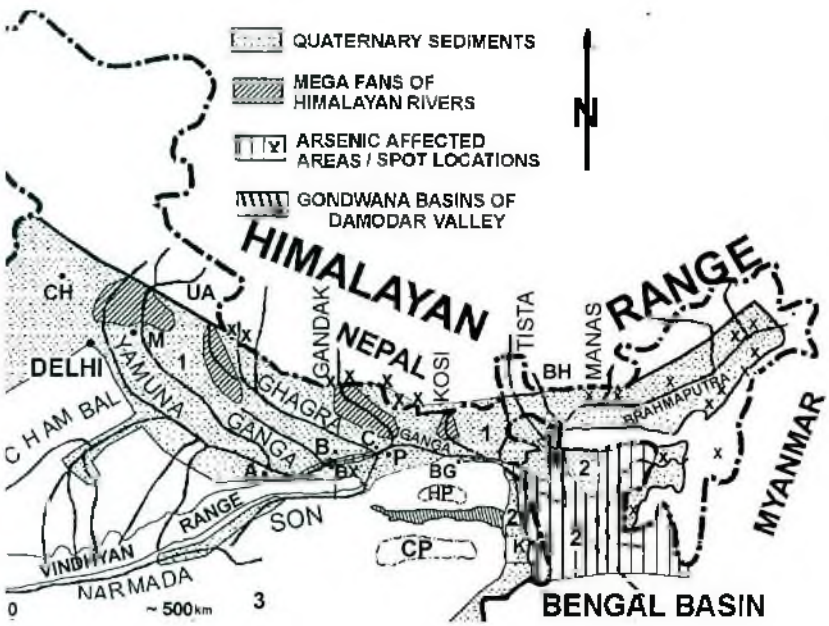


Fig No-11: Map showing the area drained by Ganga-Brahmaputra-Meghna (GBM) river system and arsenic contaminated areas [168,171].
1—Indo-Ganga-Brahamaputra Alluvial Plain,
2—Bengal Basin,
3—Dongargarh Rift Zone.

Abbreviated locality:
A-Allahabad, B-Ballia,
BG-Bhagalpur,
BH-Bhutan Hills,
BXBuxar,
C-Chhapra,
Ch-Chandigarh,
CP-Chhotanagpur Plateau,
H-Hazaribag Plateau,
P-Patna.

delta in the Bengal Basin (Fig-9). The evolution of the Bengal basin started since the Indian plate collided with the Asian plate after it broke off from the Gondwanaland in the upper Paleozonic time. The Bengal basin has developed largely over a remnant-ocean basin and the rifted eastern continental margin of India, with continental crust underlying at least the northwestern portion of Bangladesh. The Bengal basin is bounded (Figs. 10 & 11) on the west and northwest by the Rajmahal Hills (Trap), which is composed of lower Jurassic to Cretaceous trap basalts of the Upper Gondwana system. The northeast is bounded by the Garo, Khasi and Jayantia hills (west to east), which stretch for about 97 km from north to south and 240 km from east to west. In the far northeast, Shillong or Assam plateau acts as a boundary (Fig. 10). The hills and the plateaus are composed of intensely stressed Precambrian and early Paleozoic granite, gneiss, schist and quartzite overlain by the Eocene Nummilitic limestone (Fig-12).

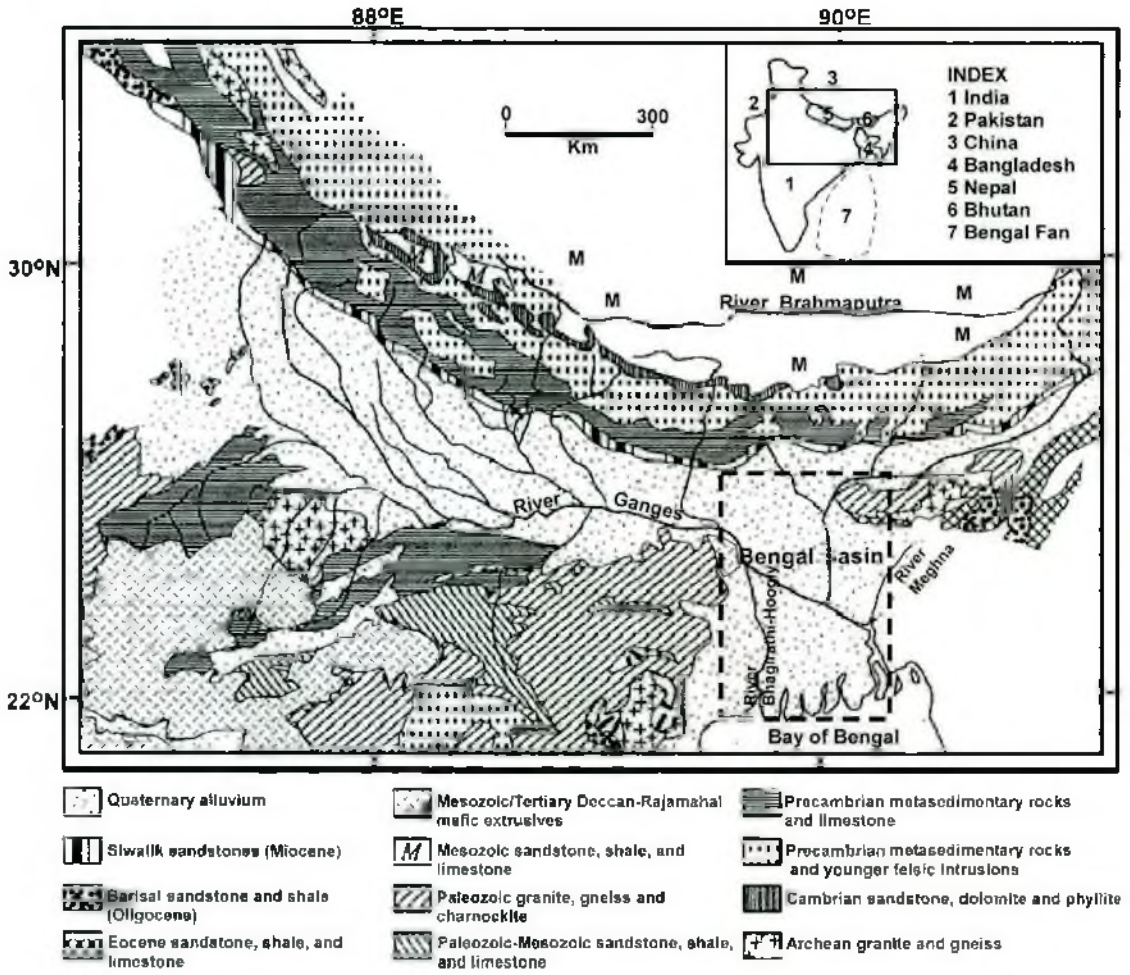


Fig No-12: Geologic map of the Indian subcontinent along with location of the Bengal basin[69]

The eastern limit of the Bengal basin is marked by the Tripura hills and Indo-Burmese fold belts to the north and Chittagong hills to the south (Fig. 10), which are composed of Paleocene–Pliocene age sediments of the Siwalik system (the Himalayan foredeep basin sediment system). More than 16 km thick synorogenic Cenozoic sediments are deposited in the basin derived from the Himalayan and Indo-Burman range. The delta in the basin extents in the south far out into the Bay of Bengal to as far as the north tip of Sri Lanka as a submarine delta know as the Bengal fan (Fig-9 &12). The basin has a thick (~22 km) Early Cretaceous–Holocene sedimentary succession that had been laid down under the influences of tectonic interactions of three plates, namely, the Indian, Tibetan (Eurasian) and Burma (West Burma Block) Plates; the dynamic of the Ganges–Brahmaputra-Meghna river systems, changes in sea levels, glacial meltdown, and other climatic influences spells of period of dry and wet periods (Fig-10, 13-18). Sedimentation within the Bengal Basin is thought to have taken place in five distinct phases and each of these sedimentation phases has been controlled by the tectonic cycles, which involved the interaction and collision pattern of the major plates (Fig-10, 13-18) and can be described as follows:

(I) Syn-rift stage (Permo-Carboniferous to early Cretaceous): started within the basins on the stable shelf (Province 1) well before the break-up of Gondwanaland during the Permo-Carboniferous to early Cretaceous period.

(II) Drifting stage (Cretaceous–Mid-Eocene): Possibly as early as 123–132 Ma, in Early Cretaceous, the break-up of Gondwanaland initiated the sedimentation phase II. During this period subsidence and marine transgression into the area of Provinces 1 and 2 of the Bengal Basin (Fig-13) had been taking place. The rate of sedimentation increased in the Santonian (about 84–88 Ma) with sediment accumulation on the continental margin of India when plate reorganization was taking placed. Most of these sediments were deposited on the northern continental margin of India, what now is the Himalayas. At this time sedimentation in the deeper part of the basin was rather slow.

(III) Early collision stage (Mid-Eocene–Early Miocene): In the Paleocene, soft collision occurred between the northern Greater Indian continental crust and the

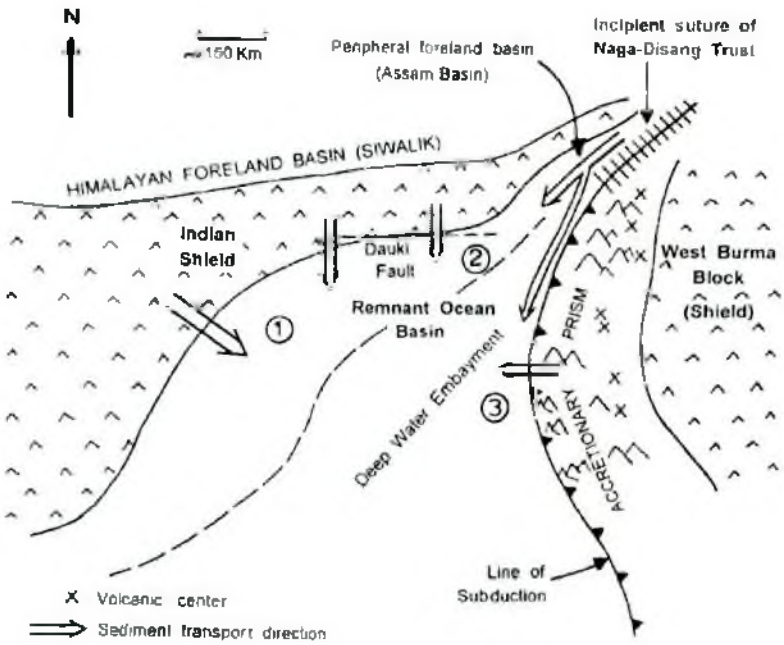


Fig No-13: Early Miocene paleogeographic representation of the Bengal Basin and surrounding region in terms of the plate tectonic model. Positions of the three geo-tectonic provinces of the basin are shown by encircled numbers: (1) The Stable Shelf; (2) The Central Deep Basin; and (3) The Chittagong–Tripura Fold Belt[170].

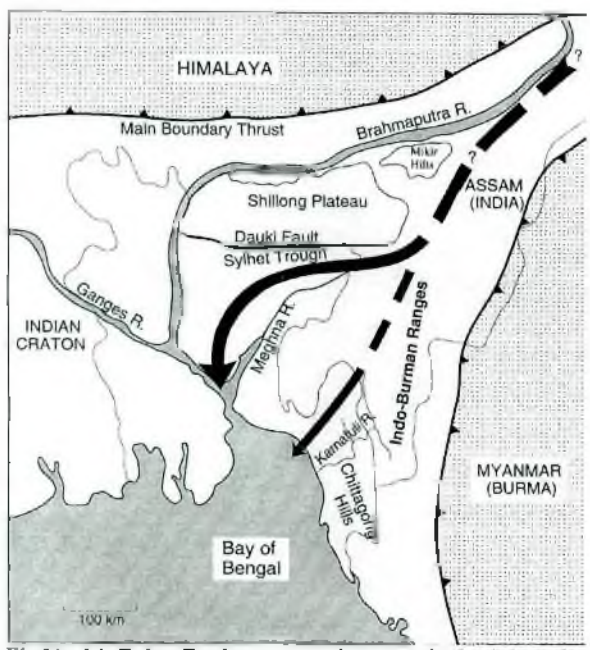


Fig No-14: **Paleo–Brahmaputra drainage** had originated close to a location of eastern Himalayan syntaxis and drains much of the Assam valley enters the northeastern Bengal basin. Deltaic deposition prograded southwestward between Himalaya and Indo–Burman orogens [165].

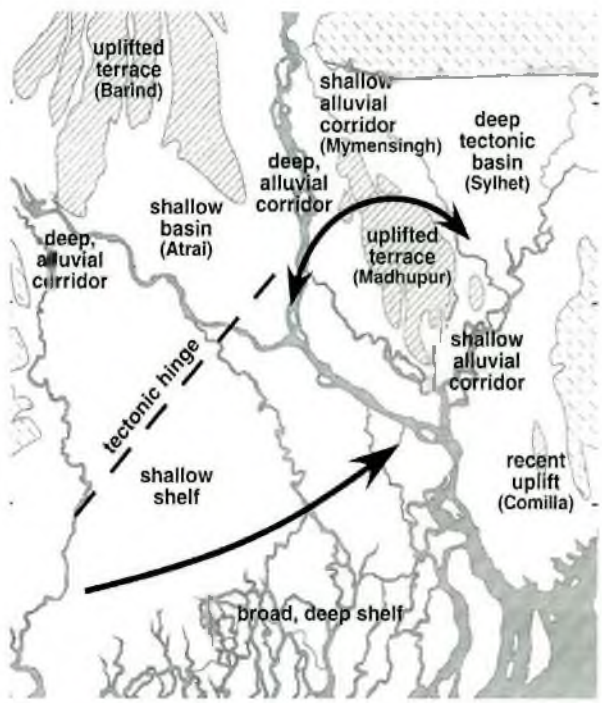


Fig No-15: Map of tectonomorphic features and controls on the Ganges–Brahmaputra delta system. Arrows show general Holocene pathways for the major river channels[166].

subduction zone lying south of Asia and this had had little or no effect on sedimentation in area what later became the Bengal Basin. During the Mid- Eocene, the beginning of sedimentation phase III, marine transgression occurred in Province 1 that was dominated by carbonate deposition, which can also be traced all along the Himalayas. During the latter half of this sedimentation phase, gradual marine regression accumulated shelfal to deltaic deposits on Provinces 1 and 2, whereas submarine fan turbidite sedimentation dominated in the southwestern portion of Province 2 and in Province 3 (Fig-13).

(IV) Sedimentation phase IV (Early Miocene–Mid-Pliocene): By Early Miocene, was initiated, the Bengal Basin took the shape of a remnant ocean basin (Fig-13), and the Provinces 2 and 3 started to act as active sediment depocenters. A major collision of the Indian plate with South Tibet and Burma plates took place in the Early Miocene, and rapid uplift occurred in the Himalayas and Tibet. At about the same time a major up thrust movement occurred along the Dauki Fault resulting in separation of the Sylhet Trough from the stable shelf, and the trough formed an important sediment depocenter. Repetitive marine transgressions and regressions dominated the depositional processes over the entire Bengal Basin during phase IV.

(V) Late collision stage (Mid-Pliocene–Quaternary): Mid-Pliocene marks the beginning of sedimentation phase V, when the final marine regression from most of the Bengal Basin concomitant with the tectonic upheaval of the eastern Bengal Basin, established a fluviodeltaic environment of deposition. With continued Plio-Pleistocene collision of India with Tibet and Burma, and rapid rise of the Himalayas and the CTFB (Chittagong–Tripura Fold Belt), the sediment depositional center was shifted further south and the present Faridpur and Hatia Troughs became the major sediment depositional centers.

It is thus apparent that sediments from the Himalayas, adjoining of India and Burma had contributed to the development of Bengal basin and the Ganges–Brahmaputra-Meghna river systems had played a significant role in addition to that of the tectonic activity and, climatic changes and accompanying sea level changes.

To have an understanding of the source of arsenic can be gained if the potential source rocks, weathering processes, paleohydrology, regional tectonic, climate evolution (mainly Holocene global warming and the retreat of glaciers), and chemical weathering need to be taken into consideration.

SOURCE OF ARSENIC DEPOSITED IN BENGAL DELTA[172-191]

A variety of anthropogenic sources have been proposed as the cause of particular occurrences of elevated arsenic concentration in groundwater in the Bengal Basin, including industrial pollution, the use of agrochemicals and wood preservatives, only a mineralogical source within the sediments of the Bengal Basin is consistent with the full regional extent and magnitude of the arsenic occurrence as observed. It is now widely accepted, that arsenic in groundwater of the region has a source within the sediments of the Bengal Basin and is of a natural origin (geogenic in nature). And its presence is related to the geogenesis of the Bengal delta. There is a consensus that the arsenic in groundwater in the West Bengal and Bangladesh was geogenic in origin. But there is a debate as to the source of this arsenic enrichment in Holocene groundwater in the region. Some refer to arsenic-bearing pyrite coming from the Gondwana coal seams and from the Rajmahal volcanics as the primary geogenic source of the arsenic found in the aquifers of Bengal delta, others had proposed the Quamdo-Simao ophiolitic province in the central-eastern part of the Tibetan plateau (located north of the Namche Barwa syntaxis) as the primary source of arsenic transported during Miocene time toward the Siwalik foreland basin. While others have proposed that though the sediments of Bengal basin had been derived from the Siwaliks which had originated from sediments derived from the Indus-Tsangpo suture zone. The Indus-Tsangpo suture zone due to the abundance of arc related rocks usually rich in arsenic and the Siwalik Neogene sediments due to the abundance of clays minerals also enriched in arsenic, while most of the Himalayan units are dominated by crystalline rocks and sediments usually poor in arsenic (<2 ppm). An agreement that is observed that arsenic rich sediments had intitially been deposited in the Siwalik and thereafter had been redistributed mainly to the deltas that that had received sediments from this region through out the timeframe they had evolved. The sediments could have been derived from the Indus-Tsangpo suture zone or the Quamdo-Simao ophiolitic

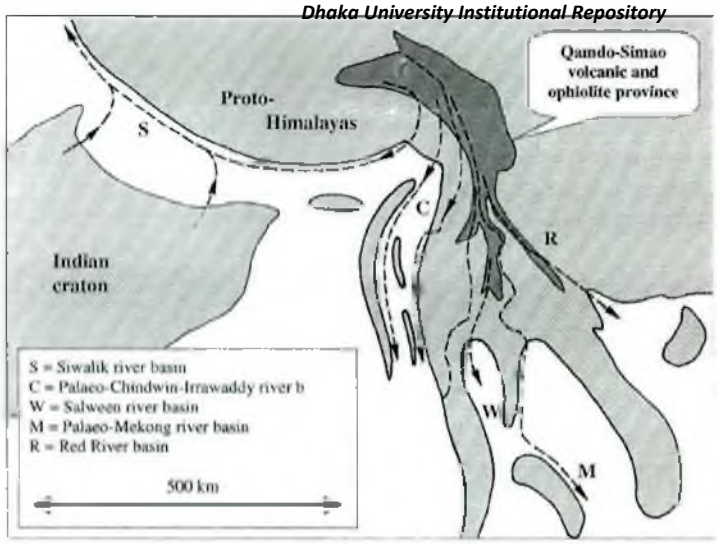


Fig No-16 : The Early Cenozoic Palaeogeography of south and south-east Asia modified and extended from Stamp in Chhibber,[178]. Note. Only the fully continental areas are shaded. Most of the adjacent (unornamented) areas were initially shallow marine, but progressively filled from headwater to downstream depositional areas. In most river basins the transgression, from marine to estuarine, swampy, lacustrine and alluvial facies was interrupted by several regressive interludes, river capture and major basinal folding.

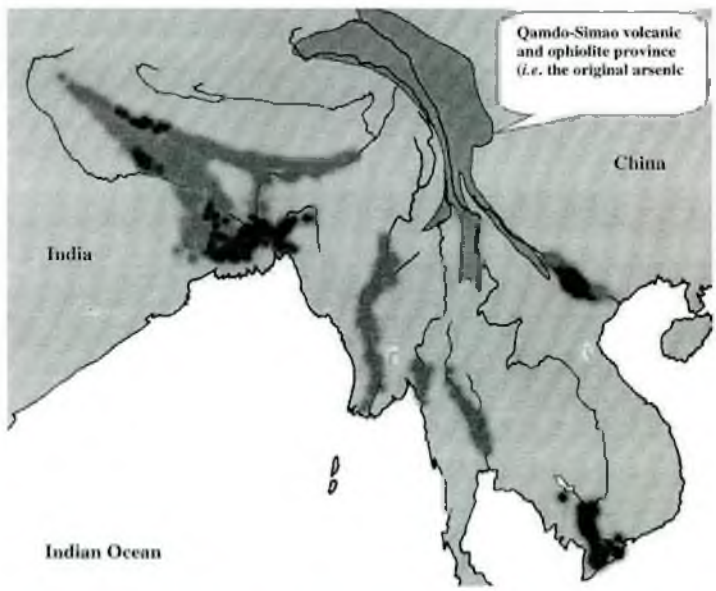


Fig No-17: The Current south and south-east Asian drainage system in relation to the Qamdo-Simao volcanic and ophiolite province, and to areas of known and suspected arsenic in groundwaters[178].

Note.
Areas of well-known arsenic-rich groundwaters are indicated by black fuzzy spots.
Areas of partially known or suspected arsenic-rich groundwaters are indicated by grey fuzzy spots.
The indicated rivers are the Ganges, Brahmaputra, Meghna, Chindwin–Irrawaddy, Salween, Mekong and Red.

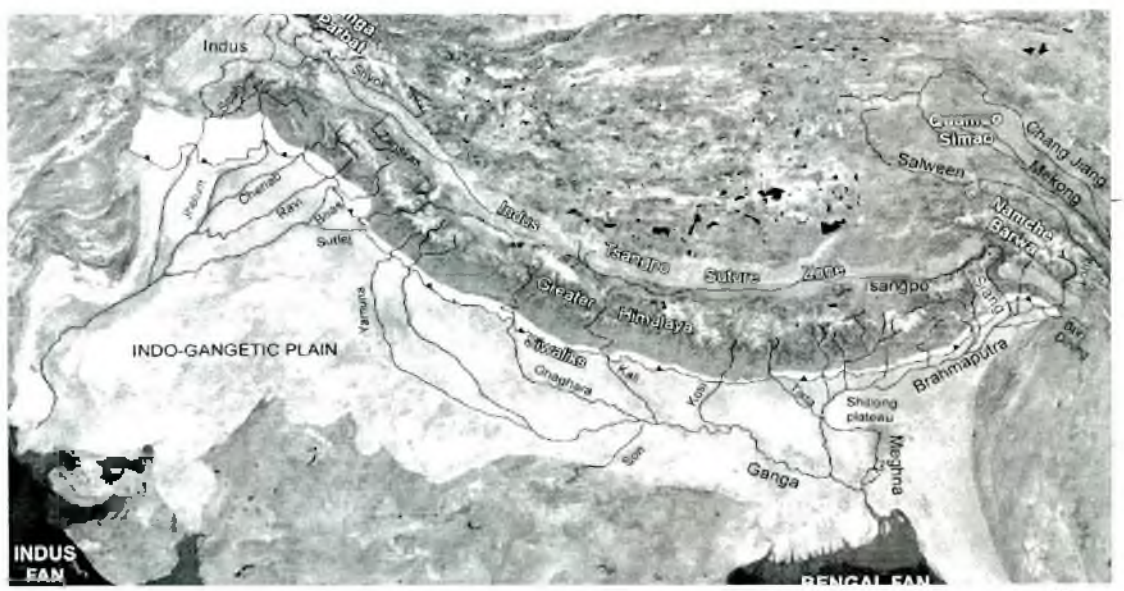


Fig No-18: Himalayan orographic map with the major drainage systems and the main Himalayan[179] geologic zones[179].

province or both. The entire process could be related to the regional tectonic dynamics; and paleohydrological evolution, climate changes (mainly Holocene global warming and the retreat of glaciers), and consequent weathering of rocks and sediments that had taken place throughout the ages.

The Qamdo-Simao link

As the Palaeo-Tethys was being consumed by subduction, obduction and Asiatic terrain-accretion to the north, the Meso-Tethyan Ocean was rapidly replacing it with thin oceanic crust in the south. This, in turn, was destroyed by further convergence as an eastward extension of the Indian–Asian collision. Further westwards during the Early Eocene (about 53 Ma) the thin Meso-Tethyan oceanic fore-basin was consumed, during the 'hard-collision' in the late Eocene (40 Ma) the rigid thick continental crust of India ploughed into similarly rigid Asiatic crust. Palaeo-Tethys was a scatter of over a dozen micro-cratonic fragments in an ocean of volcanically active oceanic sub-plates, with a complex of both constructive and destructive plate margins. Multiple accreted terranes and ophiolite-rich sutures that occur in the region east of Assam, in northern Myanmar, and particularly in south-western China, marks the extinction zone where the Palaeo-Tethys was consumed. Arsenic enrichment in this region is highly likely to have occurred through the dual processes of direct 'wrap-around' tectonic emplacement of ophiolites and associated sediments in suture zones between accreted terranes; and pre- and syn-tectonic enrichment of volatile elements as volcanic or hydrothermal emanations above subduction zones. the Permo-Triassic Qamdo-Simao (or 'Chamdo–SzeMao', a.k.a. the 'Sanjiang' area) terrain suture/volcanic province, is 'one of the gigantic metallogenic belts of the world', (shown as the darker shaded area of Figures 16 and 17), which is rich in the siderophile paragenetic host lithologies conducive to arsenic mineralization, namely ophiolitic sutures, and MORB (mid ocean ridge basalt) and para-MORB volcanics and associated oceanic sediments constitutes a likely source of arsenic. The tectonics of plate convergence, and Himalayan uplift that occurred from about the mid- to late-Eocene was accompanied by post-accretionary drainage and fluvial/sub-glacial erosion from the Qamdo-Simao province and the sub-Himalayan flood-plain basin has suffered dramatic and continuing change. Towards the close of the Eocene, when the continent–continent closure was almost

complete and Himalayan plutonic activity was well under way, fluvial sediment from the south from peninsular India was washed into the sub-Himalayan trough and the Murree formation was formed. Subsequent to the'hard inter-cratonic collision' between India and mainland Asia, the highland area was in the east, (the Qamdo-Simao province), a huge palaeo-river system, the Siwalik River was born, which during the Oligocene and early Miocene contributed towards an enormous wet tropical sediment flux that filled in the sub-Himalayan trough from east to west.

The Indus-Tsangpo suture zone link

The Indus-Tsangpo suture zone another source of arsenic in the Silwaliks that marks where the Tethys Ocean was consumed as India approached and had collided with Asia, 55 Ma ago. In the process (120 to 50 Ma) the entire N-S extent of the Tethyan oceanic lithosphere subducted along the southern margin of the Lhasa-Karakorum block giving rise to an Andean-type margin dominated by calc-alkaline plutonic rocks (mainly diorite and granodiorite with subordinate andesitic to rhyolitic volcanics), and an intra- oceanic subduction system. In the intra-oceanic paleo-subduction zone a large part had obducted giving rise to a succession of large dismembered ophiolitic units with associated ophiolitic melange marked by the abundant MORB type volcanics (pillow lava and dyke swarm) from the Kohistan arc in the west to the Namche Barwa syntaxis in the east with a 1 to 5 km thick crustal section was overlying a mantle section dominated by more or less serpentinized peridotites of mantle origin. These serpentinites had been found to be enriched in solubles elements such as As, Sb or Pb. Arsenic content reaches up to 275 ppm. Minute sulfide minerals with high arsenic in heazlwoodite (Ni_3S_3) and tinity grain of arsenide has been identified on Himalayan serpentinites. Most magnetites has been found to be of low As content (below 16 ppm) in contrast large antigorite grains (~90 ppm). The arsenic in the serpentinites from the Indus suture zone could have been concentrated in the antigorite by substitution of Si^{IV}) by As^{V}. Arsenic could also have sorbed on the magnetite surface. The arsenic-rich concentration in the serpentinites could have involved the release of As^{V} from subducted sediments to the mantle wedge beneath the volcanic arc. These serpentinites as the base of the arc acted as a sink for water and arsenic and then exposed to the surface by collision processes. The Indus Tsangpo suture zone is exposed to the surface for a long time and had been near the surface since 40 Ma. and thus had been

exposed to weathering for a long time. Consequent to the weathering processes of serpentinites leaching of Mg and Si and relative enrichment in the least mobile elements, i.e., Fe and Al had lead to the production of smectite, kaolinite, chlorite and large amount of oxyhydroxides minerals, and these oxyhydroxides can contain up to 76,000 ppm of arsenic. Thus weathering of serpentinites in the Indus Tsangpo suture zone could probably had been one of the most important sources of arsenic. Between 15 and13 Ma the Greater Himalaya was at a depth of about 10 km. The Greater Himalaya, in the western Himalaya was not exposed prior to 10 Ma, and in central Himalaya in Nepal the Greater Himalaya did not appear in the central Himalayan foreland basin until after 11 Ma or even later during the deposition of the Lower Siwalik Group. During the Miocene, the Indus-Tsangpo suture zone had feed the Siwalik foreland basin The Paleogene Himalayan foreland basin had received sediments mostly from southern Tibet and the Indus-Tsangpo suture zone that resulted from the mechanical and chemical alteration of calc-alkaline magmatic rocks, basic and ultrabasic rocks enriched in arsenic and of the metamorphic units in northwestern Himalaya. In absence of reliefs (the Himalayan barrier) to the south, the main rivers flowed directly towards the south carrying these sediments and laid them down in the foreland basin.

In the Early Miocene (after 25 Ma), the foreland basin had received sediments from the Tethyan Himalaya and 11–5 Ma, the already exposed Greater Himalaya started to be eroded and high-grade metamorphic clasts poor in arsenic, first appear in the Siwalik Group of the Himalayan foreland (Fig. 12, 13 & 18). the rivers that feed the Siwalik and Bengal bay upto 5 Ma come from the Indus Tsangpo suture zone and consequently a part of the sediments comes from As-rich sources. During the Miocene, the break-off of the Indian slab increased the altitude of the internal Himalayan belt and consequently the erosion. During this period the Greater Himalaya was extensively exposed, leading to deposition of large amount of metamorphic clasts in the Siwalik Group and the Bay of Bengal. Even though the Himalaya had become a mountain of prominence by mid Miocene, it could not have been a barrier high enough but was a youthful mountain characterized by mild relief and gentle topography.

The spectacular uplift of the Himalaya had occurred when powerful tectonic activity occurred in the interval 11 to 7.5 Ma. These tectonic events were accompanied by sudden

and drastic change in the climate conditions as result of which great volumes of detritus were eroded from the uplifting terranes and by heavy seasonal rainfalls, which found their ways to the foreland basin and to the Bay of Bengal and Andaman-Nicobar domain of Indian Ocean. After 5 Ma major changes occurred, the Himalayan rivers underwent strong reorganization. In the Indo-Gangetic plain, the major Punjab rivers (Chenab, Ravi, Beas, Sutlej) which flowed east into the Ganga river before that time were derouted into the Indus whereas in the eastern syntaxis due to the rapid uplift of the Nanga Parbat syntaxis, the Tsangpo-Brahmaputra river changed course and drained into the Brahmaputra river (Fig-11, 12, 14, 17 & 18). Moreover, the initiation of MFT located front of the Siwalik Group took place after 2 Ma with an important recent activity (shortening around 20 mm/yr from 0.3 to 0.1 Ma) leading to an important incision and erosion of the uplifted Siwalik Group.

The Siwalik link

The Siwaliks is placed over the present-day Indo-Gangetic foreland basin and is separated from the Indus-Tsangpo suture zone by the Himalayas. The Ganga plain foreland basin shows all the major components of a foreland basin system, namely an orogen (the Himalaya), deformed foreland basin deposits adjacent to the orogen (Siwalik Hills), a depositional basin (Ganga Plain) and peripheral cratonic bulge (Bundelkhand Plateau). The Siwalik formation tends to thin westwards, from about 4 km thickness in Eastern Bhutan, 3 km in Sikkim, to 2 km in the Punjab. The Siwalik Groups defines the synorogenic continental sediments of the foreland basin. They are located beneath the Gangetic plain (Terai plain in Nepal) or in the outer part of the fold and thrust belt. (Fig. 12, 13 & 15). The Siwalik Group represents a typical fluvial fining upward succession, with lower unit consisting of fluvial channel sandstone and oxidized calcareous paleosols, the middle unit is thick channel sandstones and the upper unit comprised mainly gravely braided river deposits. The base of the Siwalik Group is dated between 15.5 and 13 Ma in India and Nepal, and. the Middle and Upper Siwalik are younger than 11 Ma. Before being removed during the Holocene, sediments from the Indus-Tsangpo suture zone which is lithologically similar to the Quamdo-Simao volcanic and ophiolite province had fed the Siwalik Group during Miocene and Pleistocene time. Before 10 Ma, the Lesser Himalaya represents less than 10% of sediment input that had increased up to 30% after 10 Ma.

Activation of the MFT (Main Frontal Thrust), the most frontal one of the several north-dipping thrusts delineate tectonic boundaries in the Siwalik Group since 2 Ma had caused upliftment and consequent erosion of the Siwalik sedimentary basin. During the Plio-Pleistocene, only 20% of the motion occurred on the MFT and since the Holocene, the MFT concentrated between 50 and 100% of the whole motion. A strong activation of the MFT during the last 10,000 years had enhanced an important uplift and erosion of the Siwalik. The exposed Siwalik composed of immature argillaceous and arenaceous sediments form a 10 to 50 km wide and some 1800 km long. The Siwalik Group is probably the main arsenic reservoir in Himalaya, not the original source of this element. Combining rapid uplift of the Siwalik sediments with increasing runoff conditions during the Holocene are strongly favorable conditions to mobilize the arsenic initially scavenged in the fine-grained Siwalik clay levels and associated with iron mineralization, sulphides and clay minerals.

During the Holocene period there was alternation between high rainfall and aridity that favored weathering of the Himalayan rocks. The prolonged period of dryness in late Quaternary that included the time of Last Glacial Maximum (20,000 to 16,000 yr BP) and persisted until nearly 11,000 yr BP was followed by a climatic condition that became progressively wetter and warmer as the SW monsoon intensified, reaching its peak in the early to middle Holocene. In central Indo-Gangetic plains heavy rainfall washed away salts and carbonates from the soil and caused development of a better drainage around 8000 yr BP, in the period 7500 to 3000 yr BP there occurred higher rainfall. The wet and warm epoch was followed by a time of severe aridity around 3500 yr BP and lasting until about 2000 yr BP. The fact that during the Holocene the climate is dominated by high rainfall conditions favouring the weathering of Himalayan rocks and have enhanced fluxes of finer Fe-hydroxides minerals, clay size detritus, particularly before 3000 yr BP. This can explain the enrichment of arsenic in Holocene aquifer sediments.

The highest erosion rate varies strongly in the Greater Himalayan belt and reach peaks in the eastern syntaxis. The Siang river, draining the eastern syntaxis contributes of around 25% of total sediments reaching the Bay of Bengal, whereas the Ganga basin, which is four time large, contributes to 40% of the total sediment of the Bay of Bengal. The rest comes from Himalayan tributaries (14%) and 7% from the Shillong plateau.

During the last 10,000 years the upliftment and erosion of the Siwalik has enhanced. In the Siwalik, weathering of floodplain sediment by local rainwater had led to the formation of paleosols, downward leaching of dissolved weathering products, and precipitation of secondary carbonates nodules, Fe/Mn oxides and surrounding Fe-oxide matrix that sorbed the arsenic. The same fluids had passed through into local rivers and the weathering of flood-plain sediments had determined the elemental compositions of local rivers going to the Ganga and Brahmaputra rivers. It has been suggested that sediments of high-arsenic groundwater aquifers in the Terai plain of Nepal, Pakistan and India, are related to the eroded Siwalik. Sediments carried from the Siwalik by the minor rivers release more arsenic than those carried by major rivers from the Higher Himalaya. Eastward, the main tributaries of the Brahmaputra river (Fig-11, 12, 14, 17 & 18) cross-cut and eroded the Siwalik Group; the Siwalik appears to be the precursor source of arsenic analysed in the Brahmaputra.

The Ganga-Brahmaputra river system had mainly contributed to the buildup of the Bengal fan since almost 20 Ma. This river system had carried enormous volumes of sediments from the Himalayan belt. The average annual sediment transports by the Ganga-Brahmaputra river system is of about 1800 tons/km^2, with suspended load estimated between 540 to 1157 millions tons/yr. It is the large-river basin with the highest denudation rate on Earth (0.69 mm/yr). The sediments from the Bay of Bengal are dominated by quartz (40–55%), plagioclase (14–21%), alkali feldspar (2–10%), muscovite (illite) (2–7%), clay minerals (chlorite-illite-kaolinite) representing 4 to 10% and heavy minerals (zircon, ilmenite, rutile, amphibole and garnet). Thus, metamorphic rocks and intermediate-to-acid magmatic rocks (possibly from Himalayan region) are the original source of the sediments.

During the Holocene, particularly before 3000 yr BP the climate was dominated by high rainfall conditions that favoured the weathering of Himalayan rocks had enhanced fluxes of finer Fehydroxides minerals and clay size detritus. At present the erosion is very low in two-thirds of the basin, including the elevated Tibetan plateau, the Assam region, the Bangladesh and the Indo-Gangetic plain. Thus it is possible that the original source of arsenic was in ophiolitic, arsenic rich, arc related rocks in the Indus-Tsangpo suture zone. Climatic conditions, river flow and tectonic activity had lead to weathering of arc related

rocks followed by the transport of arsenic into the Siwalik foreland basin which had served as the arsenic reservoir from the Miocene to the Pleistocene. Intense tectonic activity in the front of the then Himalayan belt associated with high rainfall conditions during the Holocene allowed the arsenic to be remobilized, transported by the rivers and again immobilized and concentrated under oxidized conditions by Fe-oxyhydroxides and clay mineral in the Bay of Bengal.

1.9.4 SEDIMENT CHARATERISTICS

The eastern part of Bangladesh is represented by the folded bed which occupies about 18% of the country and encompasses Chittagong, Cox's Bazar, the 3 hill districts (Rangamati, Bandarban and Khagrachari), Sylhet and adjacent areas.

The Pleistocene uplands cover about 10% of the country. The flood plains of Ganges, the Atrai, Brahmaputra-Jamuna, the old Brahmaputra, the Meghna rivers covers 40% of the country including the Tista fan, Tippera Surface and Sylhet depression. The Delta complexes in the southern part cover about 32% of the country.

Generally the Bengal delta is often referred to as the "Ganges-Brahmaputra-Meghna delta" which is still active. Currently, each year in the summer monsoon season about 3 million cusecs of water and in winter about 250,000 to 300,000 cusecs of water passes through the delta into the Bay of Bengal. In addition of water about 2 to 2.4 billion tons of sediments carried by the river systems is deposited in the food plains each year. Billions of tons of sediments had been deposited in the delta over the ages, which are evident from the position of the river beds, about 7500 years bp, the river beds of the basin were about 150 m below the present level. The thickness of the sediment layer (Permian to Holocene age) is upto 20 km thick in the southern parts and about 114 m in the north.

The sediments are derived from the upland Himalayan catchments, the Indo-Burman ranges and from basement complexes of the northern and western parts of West Bengal (Rajmahal Hills, Choto Nagpur Plateau, Shillong Platueau). Along with sediments from the Ganges- Brahmaputra-Meghna catchments area many weathered minerals had entered the basin and had been deposited in the delta over thousands of years.

Major part of this sediment has been deposited by the GBM river system during Miocene to Holocene time. The oldest sediment in Bangladesh is the Premian Gondwana rock (sandstone with shale and coal beds) that overlies the Pre-Cambrian basement complex (igneous and metamorphic rock). Over the Gondwana rock successively lies the Cretaceous Rajmahal Trap (volcanic trap with sandstone and shale); Paleocene Tura sandstone (the oldest exposed rock of Bangladesh); Eocene Sylhet limestone (lime stone with sandstone beds) and Kopoili (shale with sandstone and fossiliferous beds); Oligonene Barail (sandstone); Miocene Bhuban (sandstone and shale), Bokabil (sandstone and shale) and Tipam sandstone (medium to coarse lignite bands, grey Tipam Sandstone at bottom and Grey Girujan Clay at top); Pliocene Dihing (sandstone-pebbles and laterites) and Dupi Tila (sandstone with clay contains coarse pebbles and petrified wood); Pleistocene Madhupur Clay (red clay with patches of sandstone); and Holocene alluvium (sand , silt and clay layered and inter layered with peat and vegetable matter).

In some places of the country many of the formations are missing due to depositional, non depositional and post depositional erosion.

The Quaternary sediments including the Pilo-Pleistocene are most important groundwater sources in Bangladesh. The arsenic accumulation in the Bengal Delta Plain is thought to have occurred during the late Quaternary age (Holocene age) with arsenic-containing alluvial sediments deposited by the Ganges, Brahmaputra, Meghna, and other smaller rivers that flow across the Bengal Delta Plain into the Bay of Bengal. Till today arsenic is being carried by the Ganges- Brahmaputra- Meghna river system and is being lad down in the Bengal basin. Sediments laid down in the Holocene age (0-10,000 years BP) is composed of grey clay, silt, fine sand with occasional peat and gravels; and the finer and peaty layers have high arsenic content while the coarser gravel layers contain relatively lower level of arsenic. Sediments laid down in the Pleistocene age (1.8 million-10,000 years BP) consist of reddish brown mottled clay and silt has low arsenic content. While the sediments laid down in the Plio-Pleistocene age which consists of yellowish brown fine to medium sand has very low arsenic content. It is possible that much higher levels of arsenic could have been laid down in the Bengal basin by these rivers in early geological time.

The greatest arsenic concentrations are mainly found in the fine-grained sediments especially the grey clays. A large number of other elements are also enriched in the clays including iron, phosphorus and sulphur. In Nawabganj, the clays near the surface are not enriched with arsenic to any greater extent than the clays below 150 m - in other words, there is no evidence for the weathering and deposition of a discrete set of arsenic-rich sediments at some particular time in the past.

It is likely that the original sources of arsenic existed as both sulphide and oxide minerals. Oxidation of pyrite in the source areas and during sediment transport would have released soluble arsenic and sulphate. The sulphate would have been lost to the sea but the arsenic, as As(V), would subsequently have been sorbed by the secondary iron oxides formed. These oxides are present as colloidal-sized particles and tend to accumulate in the lower parts of the delta. Physical separation of the sediments during their transport and reworking in the delta region has resulted in a separation of the arsenic-rich minerals. The finer-grained sediments tend to be concentrated in the lower energy parts of the delta. This is likely to be responsible for the greater contamination in the south and east of Bangladesh. The map of arsenic-contaminated groundwater shows that highly contaminated areas are found in the catchments of the Ganges, Brahmaputra and Meghna rivers strongly suggesting that there were multiple source areas for the arsenic.

The types of sediment deposited in the delta region have been strongly influenced by global changes in sea level during the Pleistocene glaciations [sea level was more than 100 m lower at the peak of the last Ice Age (around 18 000 years ago)]. At that time the major rivers incised deep valleys into the soft sediments of the delta. All of the highly contaminated groundwaters occur in sediments deposited since that time, while those sediments predating the low sea level stand contain little or no arsenic-contaminated groundwater.

1.9.5 CAUSE OF ARSENIC CONTANINATION IN GROUNDWATER OF BANGLADESH

The arsenic contamination in Bangladesh is of geogenic origin, sediments along with arsenic originating possibly from the Himalayan region, the Qamdo-Simao province, the Gondwana coal seams and the Rajmahal volcanics, had carried mainly by the GBM river

system aided by tectonic events and climatic factors had been laid down in this region over-time, especially in the Holocene period[175-177].

Three mechanisms as follows have been proposed to explain arsenic pollution of groundwater in the GMBD:

1. **Pyrite oxidation** [55,176,192-202]**:** It had been proposed that arsenic is present as arsenical pyrite in the alluvial sediments. And entry of atmospheric oxygen into the aquifer subsequent to aquifer draw-down leads to oxidation of arsenical pyrite and as result arsenic is released. This hypothesis had not gained popularity as despite presence of very trace amounts of pyrite in aquifer sediments, none the less the pyrites had been found not to have been oxidized. Moreover the sulfur concentrations in aquifer sediments representing both pyritic and organic sulfur do not conform to the abundance of pyrite in the sediments. Again if the arsenic would have been released in the aquifer as result of pyrite to be oxidation, the arsenic would subsequently have been sorbed to the resulting FeOOH, rather than be released to groundwater. Most important of all the groundwater in Bangladesh has been found to be anoxic rather than oxic.

2. **Anion (competitive) exchange of sorbed arsenic with phosphate from fertilizer**[175,200] **:** According to this hypothesis arsenic anions sorbed to aquifer minerals are displaced into solution by competitive exchange of phosphate anions derived from over application of fertilizer to surface soils. Phosphate derived from excessive use of phosphate fertilizer, from latrines and from the fermentation/decay of buried peat deposits and other natural organic materials may leach into the aquifer and cause displacement of arsenic from sorption sites on aquifer minerals as a result of competitive (anion) exchange resulting in arsenic pollution in the aquifer. The increase in phosphate concentration could have promoted the growth of sediment biota and desorption of arsenic from sediments, the combined microbiological and chemical process might have increased the mobility of arsenic. Considering the areal distribution of phosphorus in aquifer waters areas high in phosphorus has also been found to be arsenical; this coincidence implies that, if fertilizer phosphate or phosphate derived from natural sources could promote arsenic release in some areas of

Bangladesh. It has also been observed that concentrations of arsenic co-vary with those of phosphorus in water in certain areas (Lakshmipur) while in other areas (Faridpur) no such relation had been found. It has been suggested that competitive exchange with phosphate generated *in-situ* may contribute to arsenic pollution but this contribution is believed to be small.

3. **Reductive dissolution of FeOOH and release of sorbed arsenic to groundwater**[75,176,197,202-222]**:** At the moment it is most widely accepted that under anoxic conditions reduction of iron oxyhydroxides (FeOOH) takes place which results in release of sorbed arsenic to solution. Reduction of FeOOH is driven by microbial metabolism of organic matter. High concentrations of dissolved iron and a weak correlation between iron and bicarbonate and it is possible that additional bicarbonate could have been derived from other redox reactions, calcite dissolution and weathering of mica and feldspar, or have lost iron into precipitated phases. Concentrations of iron and arsenic have been found co-vary in aquifer sediments, but not in solution. This may be because arsenic and iron may be sequestered differentially into diagenetic pyrite and thus does not behave conservatively in solution; dissolved iron may also have been derived from weathering of biotite; and iron may have been removed from solution into vivianite, siderite, or mixed-valency hydroxycarbonates.

1.9.6 WHY SOME AQUIFERS ARE ARSENIC FREE WHILE OTHERS ARE NOT? [164,223]

Geochemical factors may also play a role since the evidence is that while the deep groundwaters are currently reducing, they are less strongly reducing than the shallow aquifers. A distinction between groundwater arsenic concentrations in the shallow and deep aquifers of the Bengal Basin exists. Differences between the sediments at depth may occur in terms of absolute arsenic concentrations and in the oxidation states and binding properties of the arsenic to the sediments. It has been suggested that the history of groundwater movement and aquifer flushing in the Bengal Basin has been important in generating the differences in dissolved arsenic concentrations between the shallow and deep aquifers. Older, deeper sediments have been subject to longer periods of groundwater flow, aided by greater hydraulic heads during the Pleistocene period when

glacial sea levels around the Bangladesh landmass were up to 130 m lower than today. Flushing of the deeper older aquifers with groundwater is therefore likely to have been much more extensive than in the Holocene sediments deposited during the last 5000–10000 years. Hence, much of the arsenic in the deep sediments may have previously been flushed away. One of the more significant 'recent' events is the global change in sea levels that had occurred over the last 130,000 years. Between about 120,000 years ago and 18,000 years ago, the sea-level steadily declined (with a few ups and downs) as glaciers expanded. The last glaciation was at a maximum some 21,000–13,500 years ago with sea levels being up to 130 m below present mean sea-level. This was a worldwide phenomenon and would have affected all of the then existing coastal aquifers. The hydraulic gradient in coastal aquifers would therefore have been much greater than at present which would have resulted in correspondingly large groundwater flows and extensive flushing. The arsenic in these older aquifers would therefore tend to have been flushed away. Between some 13,500–7000 years ago, warming occurred and sea levels rapidly rose to their existing levels. Therefore aquifers that are younger than some 7,000 years old will not have been subjected to this increased flushing that occurred during the most recent glaciation. If flushing of aquifers in relation of sea level changes are considered many of the shallow sediments in southern Bangladesh which are less than 13,000 years old, and even less than 5,000 years old, will not have experienced the extensive flushing of the last glacial period and so would likely to have higher levels of arsenic. However, deeper and older sediments, which may exceed 13,000 years old, will have been subjected to more extensive flushing. The aquifers in the Pleistocene uplifted alluvial sediments of the Barind and Madhupur Tracts which are at least 25,000–125,000 years old yield low-arsenic groundwaters, typically containing less than 0.5 µg/L arsenic and sediments in such aquifers appears to have been well flushed . At present flushing is slow because of the extremely small hydraulic gradients especially in southern Bangladesh.

1.9.7 ARSENIC IN DRINKING WATER IN BANGLADESH: EXTENT OF THE PROBLEM [52,54,55,141,154,224,225,226]

Back in the early 70s when people in Bangladesh mainly relied on surface (ponds & river) and subsurface (dugwell) water sources, diarrheal diseases and cholera were widely

prevalent. This had prompted the search of a bacteriologically safe water source. This search had lead to the tubewell initiative. With the help of a handpump and pipes with strainers sunk a few meters into the ground yielded water that was reasonably safe from the point of microbes that causes diarrheal diseases. More over this means of obtaining water became relatively cheap and in subsequent years hundreds and thousands of tubewells were installed at personal, governmental, and NGO initiatives.

The tubewell initiative to supply safe water in Bangladesh was almost a grand success, increasing number of the population with access to safe water (77.6% and 91.3% in 1991 and 1994 respectively). Though the tubewell initiative had contributed towards providing safe water from microbial point of view, a new problem surfaced. Tubewells were found to yield water containing arsenic at levels not acceptable for consumption even by the Bangladesh standard. The maximum allowable concentration of arsenic in drinking water is 0.05 mg/L which is 5 times higher than the World Health Organization provisional guideline value for arsenic in drinking water. Surveys for detection of arsenic in tubewells showed a widespread distribution of contaminated wells throughout the country. Tubewells of various proportions in 61 out of 64 districts have been found to be yielding water with containing arsenic at levels higher than the Bangladesh standard. A nationwide survey that examined 3534 water samples (8 samples per upazilla) mostly from DPHE installed tubewells located in 433 upazillas from 61 districts (excluding the 3 hill districts) found that the arsenic concentrations ranged from less than 0.00025 mg/L to 1.670 mg/L. The median and mean arsenic concentrations in the samples were 0.004 mg/L and 0.055 mg/L respectively. The mean concentration was about. 27% of the 'shallow' tubewells, (wells less than 150 m deep) exceeded the Bangladesh standard for arsenic in drinking water (0.05 mg/L) and 1% 'deep' wells (greater than 150 m deep) exceeded the Bangladesh standard. About 9% of the tubewells exceeded 0.20 mg/L, 1.8% exceeded 0.50 mg/L and 0.1% exceeded 1.0 mg/L. Based on the number of samples having an arsenic level in excess 0.05 mg/L the surveyed districts were classed as worst affected or least-affected districts. The worst affected districts were (percentage of sampled wells with greater than excess 0.05 mg/L in parentheses): Chandpur (90%), Munshiganj (83%), Gopalganj (79%), Madaripur (69%), Noakhali (69%), Satkhira (67%), Comilla (65%), Faridpur (65%), Shariatpur (65%), Meherpur (60%), Bagerhat

(60%) and Lakshmipur (56%). Arsenic concentration as high has 4.7 mg/L has been detected in water sample from Bangladesh.

1.9.8. ARSENIC EXPOSURE AND HEALTH EFFECTS IN BANGLADESH[39, 52,53,56-69,227]

Though not based on epidemiological sound surveys it has been projected based on the projected population of 125.5 million for 1999 that 35.2 million people are consuming water that contains arsenic in excess of 0.05mg/L and are at risk of health effects from arsenic exposure through drinking water, but the figure amounts to some 56.7 million when the WHO cut-off value of 0.01mg/L is considered. And since the initial detection of arsenic contamination of tubewell water in Chamagram of Baroghoria in Chapi Nawabganj district in 1993, surveys for arsenical skin lesions has lead to detection of some 10660 arsenicosis patients in 39 upazillas. The situation so far revealed is considered as the tip of the iceberg as survey of all households for arsenical skin lesions covering all arsenic affected upazillas is yet to be completed. A bigger survey conducted under the auspices of Bangladesh Arsenic Mitigation and Water Supply Project which covered 66034962 individuals residing in 12001665 households of 57482 villages in 2934 unions, reported a total of 38430 arsenicosis patients. But appropriate evaluation of such identified patients is far from complete. Many of the health problems known to be related to arsenic exposure ranging from the classical dermatological signs (melanosis, keratosis, & leucomelanosis) to respiratory problems, anemia, weakness, conjunctival congestion, diabetes mellitus, hypertension, hepatopathy, peripheral neuropathy, oedema of lower limbs, adverse reproductive outcomes, gangrene, and skin cancers are already evident amongst the arsenic exposed population in Bangladesh.

Gangrene, which is considered as one of the extreme manifestations of LEAD has been reported in a number of studies focusing on the health effects of arsenic in Bangladesh. Till date no published study is available that highlights the prevalence of LEAD amongst arsenic exposed population of Bangladesh. Against this perspective the current study had been designed to assess whether exposure to excess arsenic through drinking water in Bangladesh possess any risk difference for LEAD.

Information gained from the study when implemented can be expected to help decide if there was any need to introduce surveillance program targeting LEAD amongst arsenic exposed population. And if especial intervention program for prevention and modification of the progression of LEAD amongst arsenic exposed population in Bangladesh was required.

1.10.0. RESEARCH QUESTION

1. What proportion of arsenicosis patients in Bangladesh has LEAD?
2. What proportion of arsenic exposed population not having arsenicosis has LEAD?
3. Do arsenicosis patients in Bangladesh have a higher risk of LEAD compared to:
    a. the general population (those exposed to arsenic at levels lower than 0.05 mg/L)?
    b. asymptomatic arsenic exposed population (arsenic levels in drinking water higher than 0.05 mg/L)?
4. Does arsenic exposed asymptomatic population have a higher risk of LEAD compared to the general population (having exposure to arsenic at levels lower than 0.05 mg/L)?

1.11.0 HYPOTHESIS

H_O = Risk of LEAD amongst Arsenicosis patients is not different from that amongst those exposed to arsenic at levels lower than 0.05 mg/L.

H_A= Risk of LEAD amongst Arsenicosis patients is higher compared to that amongst those exposed to arsenic at levels lower than 0.05 mg/L.

H_O = Risk of LEAD amongst Arsenicosis patients is not different from that amongst those exposed to arsenic at levels higher than 0.05 mg/L but not having the signs of arsenicosis.

H_A = Risk of LEAD amongst Arsenicosis patients is higher compared to that amongst those exposed to arsenic at levels higher than 0.05 mg/L but not having the signs of arsenicosis.

H_O= Risk of LEAD amongst individuals exposed to arsenic at levels higher than 0.05 mg/L but not having signs of arsenicosis is not different from that amongst those exposed to arsenic at levels lower than 0.05 mg/L.

H_A= Risk of LEAD is higher amongst individuals exposed to arsenic at levels higher than 0.05 mg/L but not having signs of arsenicosis compared to that amongst individuals exposed to arsenic at levels lower than 0.05 mg/L.

1.12.0 OBJECTIVES

1.12.1 GENERAL OBJECTIVES

To assess whether exposure to excess arsenic through drinking water possess any risk difference for LEAD in Bangladesh.

1.12.2 SPECIFIC OBJECTIVES

1. To determine the prevalence of LEAD amongst the population exposed to excess arsenic and having signs of arsenicosis.
2. To determine the prevalence of LEAD amongst the population exposed to excess arsenic, but not having signs of arsenicosis.
3. To determine the prevalence of LEAD amongst the population not having been exposed to excess arsenic.
4. To compare the study groups in terms of sociodemographic characteristics.
5. To determine the influence of selected risk factors for LEAD (gender, age, obesity, smoking, blood pressure status, diabetes mellitus) amongst the different groups.
6. To determine if excess arsenic exposure had influenced in risk difference for LEAD.

1.13.0. RATIONALE

LEAD refers to progressive atherosclerotic arterial narrowing of lower limb arteries (artery) which could be asymptomatic or symptomatic, and lead to tissue loss/gangrene and even amputation(s), spontaneous or surgical [1-12]. Conventional risk factors associated with the development of atherosclerosis affecting the lower extremity vessels include age, male gender, diabetes, smoking, and hypertension[1-4, 6, 7, 10, 14, 15, 19-22, 24, 27, 28,228-232].

Arsenic has also been found to induce oxidative stress, to damage to endothelial cells, to promote apoptosis and to stimulate smooth muscle proliferation[88,90,233-238]. All these effects are known to be involved in the process of atherogenesis. Moreover diabetes mellitus[32,33,34,41,65,150,239] and hypertension[32,33,34,41,64,240], which could arise out of arsenic exposure in conjunction with the mentioned effects is believed to increase the propensity of development of LEAD. It is apparent that arsenic exposed population could be at increased risk of having LEAD compared to the normal population. Again, though abnormal cholesterol levels and lipid profile is known to be a risk factor for LEAD, study findings suggests that LEAD is correlated with ingested inorganic arsenic but not with lipid profile, and that lipid profile of those having LEAD and those not having LEAD does not differ significantly[50]. In the present study hypercholesterolemia or dyslipidemia was not taken into consideration rather it was assumed that those having hypercholesterolemia or dyslipidemia had an equal chance of being recruited in all of the study groups.

An increased risk of LEAD, as a result of drinking water containing excess arsenic has been observed in different parts of the globe[44-46,241]. Dose response relationship between peripheral vascular disease and ingested inorganic arsenic has been established and increasing risk with increasing cumulative exposure has been observed[32, 33, 35, 36, 41, 43-46, 49-50]. Though gangrene, which is considered as one of the extreme manifestations of LEAD has been reported in a number of studies[53,61,67,68] focusing on the health effects of arsenic. However till date, no published study is available so as to indicate if the arsenic exposed population is at higher risk of having LEAD compared to the general population of Bangladesh.

LEAD is considered as a marker of atherosclerotic disease throughout the body[1,2,5,6,7,11,13,24,26]. A high incidence of clinical and subclinical disease of arteries

supplying the heart and the brain had been observed amongst individuals with LEAD[1,2]. In addition to the morbidity associated with progression of LEAD to physical limitations, chronic limb ischemia, gangrene and loss of limb or part, patients of LEAD have a profound increased risk of cardiovascular ischemic events and mortality compared to the general population[1,2,5-8,10,11,13,15,19-24,27-30]. Individuals having LEAD, irrespective of being symptomatic or asymptomatic have been found to have an excessive risk of total and cardiovascular mortality[1,2,5-8,10,20,25,31].

Estimation based on the projected population of 125.5 million for 1999 shows that 35.2 million people are consuming water that contains arsenic in excess of $0.05 mgL^{-1}$ and are at risk of health effects from arsenic exposure through drinking water, but the figure amounts to some 56.7 million when the WHO cut-off value of $0.01 mgL^{-1}$ is considered[56]. This indicates that LEAD could be widely prevalent amongst the arsenic exposed population in Bangladesh, who besides having the risk arising directly out of the disease would also have an additional risk of cardiovascular ischemic events and an excessive risk of total and cardiovascular mortality than the general population.

Moreover, as preclinical cases of peripheral vascular disease (LEAD) have been found to persist even after decline or cessation of exposure to arsenic[45,51] cardiovascular consequences could be expected to be persistently higher amongst the arsenic individuals in Bangladesh having LEAD even after provision of safe water options.

The above scenario could be considered sufficient enough to justify the need to assess if the arsenic exposed population in Bangladesh faces an increased risk of having LEAD.

Symptomatic LEAD represents the tip of the iceberg of the disease burden in the population, as a substantially large proportion of the population have been found to have asymptomatic or silent disease[1-7,14,16-23]. Moreover, as individuals having either symptomatic or asymptomatic LEAD have increased risk of adverse health consequences, a sensitive tool for diagnosing LEAD would be required. Angiography (arteriography) is considered as the gold standard for detection of arterial occlusive disease[2,9,10,12,14,228,242]. But is not considered as a suitable tool for use in population based studies or for diagnosis of disease unless surgical intervention is under consideration[4,9,14,23,27,28]. Traditionally the Rose/WHO Claudication questionnaire[2,3,6-8,12,14,16,18,243] or the Edinburgh Claudication questionnaire (ECQ)[1,2,6,7,12,244] has been

widely used for screening of symptomatic LEAD and for estimation of it's population burden. The estimates of prevalence of LEAD obtained through the application of these questionnaires are considered as gross underestimates of the disease as it misses those with silent (preclinical) disease[1-8,10,12,14,16,19,21-24,27,28,228,244]. Sedentary existence or other physical conditions limiting exercise and preventing the onset of symptoms are also believed to contribute to underestimation of LEAD when claudication questionnaires were used[28]. Moreover symptoms have been considered as not to be pathognomonic for LEAD[18,22]. Recently a non-invasive technique, 'Doppler assisted ankle brachial systolic pressure index (ABI)' has found its place both in epidemiological studies and in clinical settings as well[1-14,16-18,20,23-26,228,229]. An ABI of 0.90 or less is considered to be diagnostic/indicative of LEAD[1-3,6-8,10,12,20,21,24,27-29,228,229]. ABI is considered as an efficient tool for detecting LEAD as the sensitivity and specificity of ABI for an angiographically defined stenosis of 50% or more in a major leg artery has been found to be 90% and 98% respectively[1,2,6,20,23,27,29]. Therefore the use of 'Doppler assisted ankle brachial systolic pressure index (ABI)' as a means of detection of LEAD instead of the more traditional tools in this study would allow for detection of LEAD objectively and in a broader sense as well, covering both the early and late cases.

This study when carried out would shed light as to the prevalence of LEAD in the normal population, if the arsenic exposed population were at higher risk of having LEAD and if arsenicosis patients were at higher risk compared to the arsenic exposed population not having the signs of arsenicosis.

The findings of the planned study can help health policy makers decide if there was any need to introduce surveillance program targeting LEAD amongst arsenic exposed population. And if especial intervention program for prevention and modification of the progression of LEAD amongst arsenic exposed population in Bangladesh was required.

CHAPTER II

LITERATURE REVIEW

2.1 EPIDEMIOLOGY OF LEAD

LEAD refers to atherosclerotic arterial disease of lower limbs, in which progressive arterial narrowing causes a mismatch between the oxygen supply and demand resulting in symptoms of intermittent claudication, exercise limitations, or tissue loss/gangrene[1-12].

The word claudicattion comes from the Latin word 'claudico' meaning 'limp'. Intermittent claudication refers to pain or discomfort of lower extremity (buttocks, thighs, or legs) brought on by walking or exercise and relieved by 1-5 minutes rest only to come back with exercise or walking. The discomfort is often described as aching, tightness, cramping, heaviness or numbness as well as weakness or fatigue in the muscles of the leg, thigh or buttock. The symptoms are reproducible, precipitated by the same walking duration and distance with a given speed and grade. Some are able to walk through their claudication if they walk at a slower pace[230]. Conventional risk factors associated with the development of atherosclerosis affecting the lower extremity vessels include age, male gender, diabetes, smoking, and hypertension[1-4,6,7,10,14,15,19-22,24,27,28,228,229,231,245-248]. The incidence and prevalence of LEAD are strongly age dependent and the prevalence increases with age[1-4,6,7-10,15,16,20,24,25,231,246,248]. Symptomatic LEAD represents the tip of the iceberg of the disease burden in the population, as a substantially large proportion of the population have been found to have silent (asymptomatic) disease[1-4,6,7,14,16-23]. Asymptomatic case have been found to be about 3 to 10 times as common as symptomatic disease[2,6,16,17,20,231]. Reported intermittent claudication (symptomatic LEAD) prevalence varies from 1.6 to 12% whereas rates for noninvasively determined LEAD ranges from 3.8% to 33%[24,30,231,249]. Some degree of lower limb arterial disease (atherosclerosis) has been found to be likely to be present in most of the adult population[8]. Symptomatic disease (intermittent claudication) has been found to increase by 10 fold in men from ages 30 to 44 (6/10000) to ages 65-74 (61/10000); and 20 fold in women (3/10000 to 54/10000). And the male-female ratio for all LEAD is about 1.3 to 2.7[231].

The prevalence of LEAD has been found to be approximately 3% among people younger than 60 years[9,16]. Table no-10 shows some prevalence estimates of LEAD and intermittent claudication by age. The prevalence rates for LEAD increases when more liberal criteria for symptomatic disease (Edinburgh or San Diego claudication

questionnaire) is used or when LEAD is defined by objective noninvasive criteria of ankle brachial index (ABI) <0.90[231].

Table no-10: Prevalence estimates of LEAD and intermittent claudication by age[231].

Age (yr)	IC (pooled analysis) (%)	Rotterdam Study		San Diego Study	
		ABI<0.90	IC (%)	NI test (%)	IC (%)
30-34	0.8				
35-39	0.8				
40-45	1.3				
46-49	1.3			2.5	0.0
50-54	2.0				
55-59	2.3	9	1.0		
60-64	3.1	11	1.2	8.3	2.4
65-69	5.8	15	1.7		
70-74	6.8	17	2.3	18.8	2.7
ABI =Ankle brachial index, IC= intermittent claudication, NI=noninvasive					

The prevalence rates by non-invasive testing has been reported to be 2.5% at ages of 40 to 59 years, 8.3% at ages 60 to 69 years and 18.8% at ages 70 to 79 years. And prevalence has been shown to higher in men than in women[7]. LEAD has been reported to be strongly associated with hypertension[247,250]. And 50-90% of individuals with LEAD had been reported to have hypertension[249-251].

Based on a PAD diagnosis of an ABI <0.9: Cardiovascular Health Study (CHS) had revealed that the relative risk (RR) for PAD was 4.05 for diabetes and 2.55 for current smoking in the[252-254] had reported the likelihood of an individual having LEAD (ABI<0.9) was increased by having diabetes (OR 2.5) or being a smoker (OR 1.6). Stoffers HEJH et al[255] reported that prevalence of LEAD was highest amongst the diabetic (8.7%) whereas the rate was 4.2% among those with impaired fasting glucose and 3.0% among individuals with normal blood sugar. They also reported that the LEAD prevalence among current smokers was 7.7% while in former smokers and never smokers it was 4.4% and 3.0% respectively. Novo S[256] reported that incidence of LEAD was

influenced by occupation and incidence of LEAD was higher among agricultural workers than among civil servants.

2.2 DIAGNOSIS OF LEAD

Angiography (arteriography) is considered as the gold standard for detection of arterial occlusive disease[2,9,10,12,14,228,242], but is not considered as a suitable tool for use in population based studies or for diagnosis of disease unless surgical intervention is under consideration[4,9,14,23,27,28]. Most clinicians diagnose PAD from symptoms such as intermittent claudication and rest pain, and signs such as diminished peripheral pulses, ischaemic ulceration and gangrene[1].

Table no-11: Signs and symptoms of LEAD[230]

Intermittent claudication :Exertional leg pain and relief with rest
Nocturnal or rest pain relieved on dependency
Cool or cold foot on palpation
Absent pulses
Blanching or pallor on elevation
Delayed venous filling after elevation
Dependent rubor
Atrophy of subcutaneous fatty tissue
Shiny skin
Loss of hair on foot and toes
Thickened nails, often with fungal infections
Gangrene or no healing ulcer

Traditionally the Rose/WHO Claudication questionnaire[2,3,6-8,12,14,16,18,243] or the Edinburgh Claudication questionnaire (ECQ) [1,2,6,7,12,244] has been widely used for screening of symptomatic LEAD. But the validity of intermittent claudication as a measure of underlying atherosclerotic peripheral arterial disease was considered to be doubtful. The sensitivity in detecting individuals with disruption in arterial blood flow assessed by non-invasive techniques was found to be less than 20%[12,18] but is more than 50% in detecting severe arterial occlusions demonstrated by angiography[12]. On the other hand the questionnaire was found to be highly specific (>98%) in correctly identifying healthy individuals [2,12,18]

Intermittent claudication is classically evoked by exercise, with the patient usually being able to give almost the precise distance as to its onset. Claudication pain is promptly relieved by rest in the majority of claudicants. Most claudicants describe their pain as

dull, aching, cramping, or a heaviness[31]. Moreover symptoms have been considered as not to be pathognomonic for LEAD[18,22]. The differential diagnosis for intermittent claudication includes venous claudication (history of deep venous thrombosis), chronic compartment syndrome (generally seen in athletes), spinal stenosis, osteoarthritis of the hip, as well as some less common pathologic states such as rheumatologic/ connective-tissue diseases (table no-12). In the elderly population, spinal stenosis is a common comorbid condition. The pain of spinal stenosis is more variable in onset and may occur with prolonged standing; the pain is relieved gradually by leaning forward with flexion of the lumbar spine.

Table no-12: Differential diagnosis for intermittent claudication[2,4,5]

Spinal stenosis
Venous claudication
Chronic deep venous thrombosis causing venous claudication
Chronic compartment syndrome
Osteoarthritis
Rheumatologic/connective-tissue diseases

And estimation of population burden of LEAD using questionnaires are considered as gross underestimates of the disease as it misses those with silent (preclinical/asymptomatic) disease[1-8,10,12,14,16,19,21-24,27,28,228,244]. Sedentary existence or other physical conditions limiting exercise and preventing the onset of symptoms are also believed to contribute to underestimation of LEAD when claudication questionnaires were used[12,28].

Examination of the lower extremity arterial system is often employed in the diagnosis of lower extremity arterial disease. The femoral, popliteal, posterior tibialis, and dorsalis pedis pulses are palpated and graded. Peripheral pulses are graded as 0–2, with normal pulsations being 2, diminished 1, and absent 0[2,3,257,258].

The inability to detect the pulsation of the posterior tibial artery behind the internal malleolus, or of the dorsalis pedis artery, is widely used as an important physical sign. The absence of pulses in the patient with symptoms or signs of ischaemia of the foot or intermittent claudication, is regarded as highly significant. On the other hand, the incidental discovery of an impalpable ankle pulse in an individual who does not present with an ischaemic foot is given widely varying interpretations by different clinicians. The finding may be dismissed as an observer error. The anatomical course of the artery may

be thought to be anomalous. And in older age-groups the absent pulse may be regarded as merely evidence of ageing, or it may be taken as a sign of atherosclerosis. In around 20% of the adult population at least one pulse is not detected when femoral, popliteal, posterior tibialis, and dorsalis pedis arteries are palpated[12,18]. Other than arterial disease, obesity and other factors could make detection of arterial pulsation difficult. The dorsalis pedis artery is impalpable in 0% of to 12.4% of normal limbs and the posterior tibialis pulse is in 2.8 to 12.8%[257]. The dorsalis pedis pulse is congenitally absent in approximately 10% of the population and that the posterior tibialis pulse is congenitally absent in <1% of the population[244,257]. Congenital absence of these pulses is more common in Caucasians than in blacks[244]. An abnormal posterior tibialis pulse is 71.2% sensitive and 91.3% specific for PAD, whereas an abnormal dorsalis pedis pulse is only 50% sensitive and 73.1% specific[16]. Moreover the dorsalis pedis pulse is said to be misplaced in 8% of limbs and the peroneal artery replaces the posterior tibial in 5% of cases[2,12,244,257]. An absent pulse in one or more arteries was found to be 48% sensitive and 96% specific; while diminished or absent pulses were reported to be 77% sensitive and 86% specific[12]. Moreover variability in the detection of peripheral pulses was found to be high. Overall agreement between 3 observers on the presence or absence of dorsalis pedis and posterior tibial pulses was reported as 74% and the probability that if one could not detect a pulse the other two would agree was about 0.5 which is considered to be higher than would be expected to have occurred by chance[12]. Thus for purpose of epidemiological studies palpation of the peripheral pulses could be considered as an insensitive measure of peripheral arterial disease.

Ankle systolic pressure obtained by a cuff inflated proximal to the ankle and detecting the return of blood flow by means of a Doppler probe placed over the posterior tibial or dorsalis pedis artery has been reported to correlate closely with the intra-arterial recordings of systolic blood pressure of the arteries[12]. Recently a non-invasive technique, 'Doppler assisted ankle brachial systolic pressure index (ABI)' has found its place both in epidemiological studies and in clinical settings as well[1-14,16-18,20,23-26,228,229]. An ABI of 0.90 or less is considered to be diagnostic/indicative of LEAD[1-3,6-8,10,12,20,21,24,27-29,228,229, 246,259]. ABI is considered as an efficient tool for detecting LEAD as the sensitivity and specificity of ABI for an angiographically defined stenosis of 50% or more in a major leg

artery has been found to be 90% and 98% respectively[1,2,6,20,23,27,29]. Moreover it has been reported that an ABI higher than 0.90 was almost 100% specific in identifying supposedly healthy subjects[12]. An ABI of < 0.4 represents severe arterial occlusive disease with a high potential for tissue loss and an ABI > 1.3 indicates a noncompressible calcified artery[2]. Furthermore the variability in both ankle systolic pressure and ABI due to taking measurements on different occasions by different observers has been reported to have marginal effect on between-subject variability which suggests that ankle systolic pressure and ABI could be a suitable measure in epidemiological studies[12]. None the less an abnormal ABI is considered as an important marker of atherosclerosis in other vascular beds[13,251,260].

To sum up the intermittent claudication questionnaire lacks sensitivity but is highly specific, palpation of pulses is more sensitive but less specific, whereas ABI is both highly sensitive and specific. Therefore the use of 'Doppler assisted ankle brachial systolic pressure index (ABI)' as a means of detection of LEAD instead of the more traditional tools in this study would allow for detection of LEAD objectively and in a broader sense as well, covering both the early and late cases.

2.3 SIGNIFICANCE OF LEAD

LEAD is considered as a marker of atherosclerotic disease throughout the body[1,2,5,6,7,11,13,24,26]. Besides the morbidity associated with progression of LEAD, patients of LEAD have been found to have a profound increased risk of cardiovascular ischemic events and mortality compared to the general population[1,2,5-8,10,11,13,15,19-24,27-30].

LEAD is linked to increased CAD and CBVD events[231]. The prevalence of crerbrovascular disease in LEAD patients was reported to be higher ~50 to 75%[7]. Both combined CVD morbidity and mortality and CVD mortality are increased in those with LEAD with risk ratios on the order of 2 to 6[8,30,231,249,253,260,261]. Individuals having LEAD, whether symptomatic or asymptomatic, have been found to have an excessive risk of total and cardiovascular mortality[1,2,5-8,10,20,25,31,231,246].

In a follow-up study investigating the relationship between PAD and mortality from all causes, mortality rates of 61.8% after 10 years were reported for men with PAD, compared with 16.9% in normal men[5]. The corresponding mortality rates for women

were 33.3% and 11.6%, respectively. Less than a quarter of patients with severe symptomatic PAD survived 10 years[252,253].

Whether symptomatic or asymptomatic individuals with LEAD have 20-40% increased risk of nonfatal MI, 60% increased risk of progression to CHF and 90-500% increased risk of fatal MI and CAD death[231]. An abnormal ABI has been found to be an independent predictor of cardiovascular mortality (RR=4) and cerbrovascular events (RR=2)[262,263]. An ABI of <0.9 has been found to be associated with a 2.4 fold higher mortality rate and a 2 fold higher cardiac event rate[7].

2.4 ARSENIC AND LEAD

A series of epidemiologic studies have shown that exposure to drinking-water arsenic was associated with the development of lower extremity arterial disease (LEAD) often known as BFD. Such evidences are discussed below.

EPIDEMIOLOGICAL EVIDENCES

Lower extremity arterial disease (Peripheral vascular lesions) has been reported in different arsenic exposure situations which includes exposure via drinking water. There are reports of increased occurrences vascular diseases

Vascular effects were described by Geyer in 1898 among residents of Reichenstein mining town in Silesia (Poland) where the population had been exposed to arsenic via contaminated drinking water. For several centuries a significant segment of the population suffered from the disease known then as Reichstein disease, a chronic form of arsenicism (arsenicosis). An increased occurrence of gangrene –"Altersbrand" among this population had been reported[37,40]. Peripheral arterial disease had been reported from wine making regions of Palatinate and the Moselle area of Germany, and from Austria. In Palatinate among 80 cases of arsenicosis detected during the period 1935-1945, 13 had severe peripheral arterial disease requiring surgical amputation. In around 1939, in Moselle area out of 180 vintners with arsenicosis, 41 cases (~23%) were identified as having peripheral vascular lesions. Fifteen (15) were clinically diagnosed as having peripheral disease and among them 6 had gangrenous lesions. Most of these cases were reported to be moderate to light smokers and, "house drink" and wine was identified as the main exposure source of arsenic[37,40,41]. Back in 1976, peripheral vascular lesions has

been reported among a group of 100 vinedressers, who were examined over 30 years after the arsenic exposure had been terminated, the average duration of exposure of this group was 20 years. Symptoms and signs of endarteritis obliterans and acrodermatitis atrophicans was reported to be in between 60% and 95% among those in various age groups from 50 to 80 years of age. A case of amputation among a non smoker vintner was reported. These symptoms were found in only 1-2% of a control group that had not been exposed to arsenic. Contaminated wine was stated as the most important source of arsenic exposure[264]. An increased risk of peripheral vascular disease as a result of drinking arsenic contaminated water has been reported from Taiwan, Chile, Mexico and China. Peripheral vascular disease was also reported in inhabitants of the Antofagasta region of northern Chile, who had been exposed to arsenic levels of about 0.6 mg/litre in the drinking water for 15 years. A clinical investigation among 180 inhabitants revealed several effects associated with chronic arsenic exposure, including hyperkeratosis, and a high prevalence of cardiovascular disturbances. Most common were peripheral vascular phenomena, i.e., Raynaud's syndrome and acrocyanosis, which were present, respectively, in 38.8% and 24.3% of the persons with abnormal skin pigmentation attributed to arsenic exposure, compared with 9.3% and 12.5% of persons with normal skin. Infants and children showed more pronounced symptoms than adults. Cases of peripheral vascular disease requiring amputations had also been reported [40,41,241].

Arsenicosis has been reported from the rural parts of the Region Lagunera located in northern Mexico. Among the arsenic exposed population of the area 4% were reported to have peripheral vascular alterations in different stages of severity that had led to amputations in 0.7% of the cases[265]. Arsenic exposure through drinking water and concomitant arsenicosis has been reported from Cordoba and Salta provinces of Argentina. Though overt Peripheral vascular disease, gangrene or amputations had not been reported from these areas angiographic study of lower extremities of 12 arsenicosis patients had demonstrated microangiopathies with obstruction of terminal arteries. Among these individuals 66% were less than 50 years of age and half of them were less than 25 years of age[40].

Blackfoot disease (BFD) in connection with arsenic exposure, referenced to in this study as LEAD, has been extensively studied in Taiwan. A large proportions of these

studies/reports are not directly accessible as they are had been published in journals only available locally in Taiwan or had been generated as reports for different agencies in Taiwan. "Blackfoot disease (BFD)" refers to an endemic peripheral vascular disease (PVD), locally known as "Wu Chiao Ping" that leads to progressive gangrene of the legs has been known in Taiwan since the 1920s. It had increased in prevalence since the 1950s[47,49,266,267]. This condition is characterized by an insidious onset of coldness and numbness in one or both feet, progressing on to ulceration, black discolouration and gangrene in the toes and feet and occasionally in the fingers. The induction period of the condition (BFD) was found to be about 20-30 years and the time required for progression to actual gangrene varied from a few months to several years. The progression of the disease could be intermittent with periods of remissions. Onset of by exacerbation of the condition and of new cases was frequently found to be associated with cold weather and injuries. At onset affected individuals experienced numbness, coldness and blanching of toes and occasionally in the fingers. Pulses of dorsalis pedis and posterior tibial artery of the affected limb(s) was often weak or absent. Affected individuals often experienced painful intermittent claudication. Pain, often excruciating (lancinating, gnawing or burning) ensued before or was coincident with the appearance of a small gangrenous lesion. The gangrene after beginning in the distal portion of the toes extends centrally and demarcates at one of the several levels with eventual spontaneous or surgical amputation. It has been reported that the progression of the disease could be intermittent with periods of remissions. It was observed that generally a period of 1 month to 2 years lapses between the appearances of first sign of gangrene. A wide variation in the pattern of involvement has been reported, and generally one foot is affected before the other[32 37,40,41,43,45,47,49,50,268,269]. Sporadic cases of BFD occurred as early as in the early 20th century. However, not much attention had been paid until after the mid-20th century when cumulative case numbers became noticeable. . Peak incidence of BFD was found between 1956 and 1960, with prevalence rates ranging from 6.51 to 18.85 per 1000 population in different villages. The disease was found to afflict arsenic exposed individuals in a wide range of ages, from 2 to 87 years, and the mean age of onset was 52 year. The sex ratio for men to women was 1.5:1. Sporadic cases were noted since the early 20th century[269,267,270]. Arteriographic evidence of marked narrowing in the lumina

of major arteries of the affected leg or arm; and areas of complete obstruction and development of collateral arterial networks in BFD patients has been reported by Tseng et al (1961)[271]. Histologically thromboangitis obliterans and arterioscleriosis obliterans has been observed[272]. About 30% of the BFD patients had histologic lesions compatible with thromboangiitis obliterans and 70% showed changes of arteriosclerosis obliterans. The common histological features in the thromboangiitis obliterans group include 1) fibrinoid necrosis of the whole vessel wall of arterioles, venules or precapillaries; 2) proliferation and activation of vascular endothelium; and 3) diffuse infiltration of inflammatory cells throughout the whole vessel wall, giving rise to a picture of nodular non-suppurative, non-thrombotic panarteritis. Histological features of vessels found in arteriosclerosis obliterans group include 1) thrombus formation with newly formed vessels having reduced number and size, leaving a poorly vascularized, hyalinized dense fibrous tissue like an old scar tissue; 2) intimal sclerosis; 3) medial calcific change; and 4) adventitial change of periarterial fibrosis. Thickening and fibrinoid degeneration of the blood vessel wall and perivascular small round cell infiltration of the affected skin in early stage of blackfoot disease has been described. In the gangrenous limb proliferation and dilatation of the dermal vessels and occlusion of subcutaneous arterioles with thrombosis and proliferation of their vasa vsorum has been observed[40,270].

Ch'i IC & Blackwell RQ (1968)[47] carried out a controlled retrospective study in a blackfoot endemic area located along the western coast of Taiwan to detect any possible new factors which might either contribute toward or precipitate the onset of the disease and make a quantitative study of the suspected factors particularly deep well water. The study was carried out on 353 Blackfoot subjects (203 male and 150) and matching 353 controls. In the study Blackfoot subjects were included in three categories: 1).amputated Blackfoot subjects-those having amputation (spontaneous or surgical); 2) probable Blackfoot subjects- registered Blackfoot subjects having two or more of the signs or symptoms Blackfoot disease that included numbness, coldness, pain, intermittent claudication, localized heat, pallor, rubor, cyanosis, atrophic changes, ulceration or gangrene; and 3) possible Blackfoot subjects- registered Blackfoot subjects who in the past have the above listed complaints but at the time of the study exhibited no signs of the

disease and had no definite characteristic symptoms. The controls were matched to Blackfoot subjects by sex, age and districts. And controls were from households free of the disease. The Blackfoot subjects and were found to have lived in the endemic area for comparable periods before onset. In the study a striking difference between Blackfoot subjects and control subjects in the past history of principal source of drinking water was found. A significantly larger proportion of Blackfoot subjects consumed deep well water for more than 10 years before the disease onset ($p<0.001$). The relative risk for those in the Blackfoot group compared to those in the control group with respect to longer consumption of deep well water was 2.58 times as great. Ch'i IC & Blackwell RQ carried out subsequent analysis on 352 Blackfoot subjects and 233 control subjects whose principal drinking water source was deep well water. The groups were similar in sex, age, residence and year of onset. The socioeconomic level of the Blackfoot group was found to be lower than that of the control group at the time of survey, and proportion of subjects with no formal schooling was significantly higher in the Blackfoot group. In respect to past diet histories in both groups many of the diets were marginal and no marked differences between the Blackfoot and Control groups was detected, moreover factors like occupation, exposure or non-exposure to sea water during working hours, and wearing or non-wearing of shoes during working hours were not found to be associated with Black foot disease. Cigarette smoking was also not found to be associated with the development of Blackfoot disease. It was concluded that arsenic, primarily from deep well water was the likely etiologic factor in Blackfoot disease.

Tseng WP, Chu HM, How SW, Fong JM, Lin CS and Yeh S. (1968)[273] in a study based on the findings a general survey completed in 1965 and encompassing the entire population of 40421 from 37 villages in the endemic area of Taiwan. Prior to 1956 the population in these villages had used water from artesian wells for some 40 years. In the villages surveyed the arsenic content of the well water ranged from 0.01 to 1.82 ppm (measured by Natelson method); most of the well water had an arsenic content around 0.4 to 0.6 ppm. Among the population a total of 360 Blackfoot disease cases were detected. The overall prevalence of Blackfoot disease was reported as 0.9-1.2% in males and 0.7% in females. After age 39 the prevalence rates for males were significantly higher than for females. And the prevalence rose steadily until age 70 for males and 60 for females. The

prevalence rates for Blackfoot disease revealed clear-cut relationships between the condition and the arsenic content of the water in the artesian wells. The greater the arsenic content, higher was the prevalence.

Yeh S (1973)[274] reported of a follow-up study initiated in 1966, on 454 arsenical skin cancer and 360 Blackfoot disease subjects detected in endemic areas of Taiwan till 1965. The overall prevalence rates for Blackfoot disease (peripheral vascular disorder resulting in gangrene of extremities, especially the feet) was 8.9 per 1000, and the male-to-female ratio 1.99:1. A significant association between Blackfoot disease and hyperpigmentation, keratosis and skin cancer was found. Over the subsequent 5 years 422 of these patients were examined once a year, the follow-up was in fact included 344 Blackfoot disease cases, among which 229 were men and 115 women. The total population of the endemic area surveyed at the time of analysis was 54018. The mortality rate in Blackfoot disease patients was reported to be higher than that in the general population of the endemic area, especially in the total age group below 49 years. An analysis of the causes of death upto 1971 was carried out and it also included 110 Blackfoot disease cases; for comparison analysis of the causes of death in the general population of the endemic area was also done. The most common cause of death in the Blackfoot disease patients was cardiovascular disease (18.2%), followed by cancer (13.6%) and Blackfoot disease (12.7%). On the other hand cardiovascular disease deaths, cancer deaths and Blackfoot disease deaths accounted for 10.8%, 17.5% and 0.7% of the causes of death in the general population (1963-1964) of the endemic area.

Tseng WP (1977)[43] reported the findings of a house-to-house survey which by the end of 1965 covered the entire population of 40421 living in 10 villages of arsenic endemic area of Taiwan The survey was aimed in identifying arsenic exposure related skin lesions (melanosis and keratosis), and cancers in the entire population. Blood pressure measurement of persons over 20 years of age; examination of pulsation of dorsalis pedis and posterior tibial arteries; and evidence of any peripheral vascular disorder was sought. In the study Blackfoot disease diagnosis was based on: 1) objective signs of ischemia – absence or diminution of arterial pulsations, pallor on elevation or rubor on dependency of ischemic extremities, and various degrees of ischemic changes of skin, and 2)

subjective symptoms of ischemia – intermittent claudication, pain at rest, and ischemic neuropathy. The survey detected 1108 Blackfoot disease individuals of whom 669 were males and 439 females. In a subsequent follow-up in 1975, of them 528 (47.7%) were found to have died; an overall prevalence of 0.9- 1.1% for males and 0.7% for females was found; after the age of 40 the rates for males were found to be significantly higher than for females; and the prevalence rose steadily until age 70 for both sexes. And the overall male-to-female ratio was 1.3 to 1.0. In reference to arsenic concentration in well water the greater the arsenic content, the higher was the prevalence of the Blackfoot disease. Teng WP further reported that the mean age of death of the 528 Blackfoot disease patients who had died in the period between the initial survey and the follow-up was significantly lower in areas of high arsenic content than in areas of low arsenic content of the well water. Tseng WP concluded the association between melanosis, or skin cancer was not coincidental or chance occurrence, and the finding had strengthened the likelihood of a causal relationship between Blackfoot disease and chronic arsenicism, and that Blackfoot disease was part of the entity of chronic arsenicism.

Chen C-J, Wu M-M, Lee S-S, et al (1988)[268] in a case-control study on study participants (241 Blackfoot disease cases and 759 controls) drawn from four neighboring townships- Peimen (186 controls & 54 cases), Hsuechia (285 controls & 99 cases), Putai (167 controls & 54 cases), and Ichu (121controls & 34 cases) are located on the southwest coast of Taiwan to 1) to explore the possible risk factors of BFD in addition to artesian well water, 2) to assess the effects of multiple risk factors on the development of BFD, and 3) to examine the mortality from malignant neoplasms of internal organs and vascular diseases among BFD patients. Prevalent, rather than incident, subjects were recruited for the patient group in the study. The cases and controls were age, gender and residence matched. A structured questionnaire was used to obtain information on the possible risk factors of BFD. Risk factors that were explored included socio-demographic characteristics, occupational history, duration of sunshine exposure at work, residence years in various villages during the lifetime, types and duration of drinking water since early childhood, family history of BFD, history of cigarette smoking and alcohol drinking, food consumption frequency 30 years previously, and previous history of major diseases including diabetes, hypertension, heart disease, stroke, cancers, and

liver diseases. In the study one-week consumption frequency, rather than the quantity of various consumed food items, was explored. The food consumed by residents in the BFD-endemic area was simple and scarce, so the food items questioned included staple food (rice and dehydrated sweet potato chips), vegetables, fruits, eggs, pork, chicken, and fish. Exact nutrient intake was not taken into account in the study. In the study cases were those who had: 1) objective signs of ischemia (absence or diminution of arterial pulsations, pallor on elevation or rubor on dependency of ischemic extremities, and various degrees of ischemic changes in the skin); and 2) subjective symptoms of ischemia (intermittent claudication, pain at rest, and ischemic neuropathy). The cases and controls were comparable with regards to residence, age, and sex. In regards to formal educational status, occupational sunshine exposure, the duration of artesian well water consumption, arsenic poisoning, and family history of BFD, BFD patients had less formal education and more exposure to sunshine for more than 6 hours/day than did controls. Artesian well water consumption was positively associated with BFD in a dose-response relationship. Artesian well water consumption and arsenic poisoning was found more frequently in BFD patients than in controls, and family members of BFD patients were more likely to be affected with BFD than were those of controls. There was no significant difference in cigarette smoking and alcohol drinking between BFD patients and controls. However, BFD patients were less well-nourished than controls. BFD patients consumed more dehydrated potatoes and less meat, eggs, and vegetables than did healthy controls. Multiple logistic regression analysis showed that artesian well water consumption, arsenic poisoning, familial history of BFD, and undernourishment was significantly associated with the development of BFD. The multivariate adjusted odds ratios were 3.0 and 3.5 for those who consumed high-arsenic artesian well water for duration of 1–29 and ≥30 years, respectively, compared with those without arsenic exposure after adjustment for dietary factors, family history of blackfoot disease, and arsenic-induced skin lesions.

Tseng WP (1989)[49] examined a total of 1300 Blackfoot disease patients from the endemic area in Taiwan between 1958 and 1982 and followed them until 1987. The criteria for diagnosis depended upon: 1) objective signs of ischemia (absence or diminution of arterial pulsations, pallor on elevation or rubor on dependency of ischemic

extremities, and various degrees of ischemic changes in the skin); and 2) subjective symptoms of ischemia (intermittent claudication, pain at rest, and ischemic neuropathy). Of the total 1300 Blackfoot disease patients 774 were males and 526 females, the male /female ratio was 1.5:1. In more than half of the cases the symptoms had been first observed in between the age of 40 and 69 years, and in 27.9% cases the age of onset of symptoms was less than 39 years. The disease onset was generally insidious, and in some cases the onset was quite sudden. It almost always began with numbness or coldness in one or more extremities, usually the feet, and ultimately rest pain developed and progressed to gangrene. Lower extremity involvement in Blackfoot disease was observed in 97.7% of the cases. In villages where the arsenic concentrations in well water were < 0.30 mg/l, 0.30-0.59 mg/l and over 0.60 mg/l, the prevalence of BFD for residents aged 20-39 years were 0.5%, 1.3% and 1.4%, respectively; for residents aged 40-59 years, 1.1%, 3.2% and 4.7%, respectively; and for residents aged over 60 years, 2.0%, 3.2% and 6.1%, respectively. A dose-response relationship between Blackfoot disease and the duration of water intake was reported, duration of intake of arsenical water and degree of arsenic concentration were found to be related to higher frequency of the disease. Spontaneous or operative amputation was reported in 68% of the patients. Case fatality rate reported was 66.5% during the 30 years, and 44% of the deaths were reported as cardiovascular deaths.

Lin and Yang (1988)[276] carried out a case–control study on urinary arsenic level and blackfoot disease. The study included 20 blackfoot disease patients and 20 unaffected controls recruited from arseniasis-endemic areas in southwestern Taiwan, In this study the diagnosis of Blackfoot disease was undertaken by clinical evaluation and arsenic exposure was determined by urinary arsenic. The urinary arsenic level was higher in patients than controls showing an age–sex-adjusted odds ratio (95% confidence interval) of 1.7 (0.8–3.5) for those with a urinary arsenic level in 75th and higher percentiles compared with those with a level in the 25th and lower percentiles as the reference. As urinary arsenic level is a short-term biomarker of arsenic exposure, the causal temporality between arsenic exposure and blackfoot disease in this study remained to be elucidated.

Wu MM, Kuo TL, Hwang YH and Chen CJ (1989)[275] in an ecological study through an analysis of age adjusted mortality rates examined the dose response relation between

ingested arsenic levels and risk of cancers and vascular diseases among residents in the Blackfoot endemic area in the southwestern coast of Taiwan. In the study the arsenic in well water determined in 1964-1966 in 42 villages of the study area and the mortality and population data for the period 1973 -1986 available with the local household registration offices and Taiwan Provincial Department of Health was used. Peripheral vascular disease diagnosis was made by use of underlying cause (ICD 440–448) in death certificates. There was no individual measurement of arsenic exposure in this ecological study. Age-adjusted mortality rates from various cancers and vascular diseases by sex using the 1976 world population as the standard population. Among males there were 363, 230, and 136 vascular deaths and among females there were 320, 226, and 93 such deaths in villages with median arsenic levels of well water <0.30 ppm, 0.30-0.59 ppm and ≥0.60 ppm, respectively. A significant trend ($p<0.05$) in age-adjusted mortality rates (per 100,000) for all vascular diseases (for males 364.10, 421.47, and 572.68 and for females 277.5, 370.79, and 386.41 in villages with median arsenic levels of well water <0.30 ppm, 0.30-0.59 ppm and ≥0.60 ppm, respectively), for peripheral vascular diseases (for males 22.54, 57.80, and 60.40 and for females 18.20, 48.20, and 35.82 in villages with median arsenic levels of well water <0.30 ppm, 0.30-0.59 ppm and ≥0.60 ppm, respectively) and cardiovascular diseases (for males 125.87, 153.98, and 259.51 and for females 91.14, 153.07, and 144.74 in villages with median arsenic levels of well water <0.30 ppm, 0.30-0.59 ppm and ≥0.60 ppm, respectively) was observed . The age-adjusted mortality from PVD per 100,000 persons for the arsenic level of <0.30, 0.30–0.59, and ≥0.6 ppm was 22.5, 57.8 and 60.4, respectively, for males (P for trend <0.01) and 18.2, 48.0 and 35.8, respectively, for females (P for trend <0.05). The authors concluded that there was significant dose-response pattern of association of arsenic levels in well water with peripheral vascular diseases and cardiovascular diseases, but no such association for cerebrovascular accidents was detected.

Tseng CH, Chong CK, Chen CJ, et al (1996)[45] carried out a cross-sectional study involving 582 subjects select from a cohort selected for a long term follow-up study on health hazards associated with long term arsenic exposure, to examine the correlation between previous arsenic exposure and peripheral vascular disease after stopping consumption of high-arsenic artesian well water for more than two decades in blackfoot

disease endemic villages in Taiwan.. All of the 582 subjects (263 were men and 319 women) were adults and their mean age was 52.6 ± 10.years.. All participants underwent Doppler ultrasound measurement of systolic pressures on bilateral ankle (posterior tibial and dorsal pedal) and brachial arteries and estimation for long-term arsenic exposure. The diagnosis of peripheral vascular disease was based on an ankle-brachial index (the ratio between ankle and brachial systolic pressures) <0.90 on either side. Risk factors for PVD were also collected from study subjects. They included age, sex, body mass index, cigarette smoking, disease status of diabetes mellitus and hypertension, and serum lipid levels including total cholesterol and triglyceride. Three indices of arsenic exposure were estimated: 1) duration of living in blackfoot disease endemic villages; 2) duration of artesian well water consumption; and 3) cumulative arsenic exposure in mg/l-years based on the detailed history of residential addresses and artesian well water consumption and the arsenic concentration in artesian well water. Age was found to be associated with an increasing prevalence of PVD in both genders, and women had a two-fold higher prevalence of PVD than men in all age groups. All the indices of long-term arsenic exposure were significantly associated with prevalence of PVD showing a dose-response relation. The odds ratios (95% confidence intervals) after adjustment for age, sex, body mass index, cigarette smoking, serum cholesterol and triglyceride levels, diabetes mellitus and hypertension were 2.77 (0.84-9.14) and 4.28 (1.26-14.54) for those who had cumulative arsenic exposure of 0.1-19.9 and ≥20.0 mg/l-years, respectively, compared with those who were not exposed. The study found that the risk of PVD increased with increasing cumulative exposure to arsenic, with a statistically significant increase for the high subgroup (≥20 (mg/litre) year). This association persisted when different cutoff points for ABI were used to diagnose PVD. The study suggested a close relation between long-term arsenic exposure and peripheral vascular disease in blackfoot disease endemic villages in Taiwan, and that the problem persists among those who have had a long term arsenic exposure exposed even after stopping consumption of the contaminated water. This study used a more objective and sensitive tool to identify PVD as well as an individual measure of arsenic exposure.

Tsai et al. (1999)[277] in an ecological study conducted in the arseniasis-endemic areas in southwestern Taiwan, mortality from PVD during 1971–1994 was compared with those

of general populations in southwestern and entire Taiwan. Underlying cause (ICD 440–448) in death certificates was used as the basis for the diagnosis of peripheral vascular disease. There was a significantly increased age-adjusted mortality from PVD with a standardized mortality ratio (95% confidence interval) of 3.56 (2.91–4.30) for males and 2.30 (1.78–2.93) for females compared with residents in non-endemic areas of southwestern Taiwan.

Wang et al., (2001)[278] in a case–control study that included 31 patients and 30 unaffected controls from arseniasis endemic areas of southwestern Taiwan, examined arsenic in arterial tissues and risk of blackfoot disease. The diagnosis of Blackfoot disease was made by clinical evaluation. Blackfoot disease patients were found to have higher arsenic contents in arterial tissues than unaffected controls. The crude odds ratio (95% confidence interval) was 2.4 (2.0–2.9) for those with arsenic contents in arterial tissue in the 75th and higher percentiles compared with those with contents in the 25th and lower percentiles as the reference.

Tseng CH, Huang YK, Huang YL, Chung CJ, et al (2005)[279] in a cross sectional study in the blackfoot disease (BFD) - hyperendemic area in Taiwan examined long-term exposure to ingested inorganic arsenic is associated with peripheral vascular disease (PVD). The study further examined the interaction between arsenic exposure and urinary arsenic speciation on the risk of PVD. A total of 479 (220 men and 259 women) adults residing in the BFD-hyperendemic area were studied. Doppler ultrasound was used to diagnose PVD (ABI <0.90 on either side). Arsenic exposure was estimated by an index of cumulative arsenic exposure (CAE). Urinary levels of total arsenic, inorganic arsenite (As^{III}) and arsenate (As^{V}), monomethylarsonic acid (MMA^{V}), and dimethylarsinic acid (DMA^{V}) were determined. The association between PVD and urinary arsenic parameters was evaluated with consideration of the interaction with CAE and the confounding effects of age, sex, body mass index, total cholesterol, triglycerides, cigarette smoking, and alcohol consumption. Results showed that aging was associated with a diminishing capacity to methylate inorganic arsenic and women possessed a more efficient arsenic methylation capacity than men did. PVD risk increased with a higher CAE and a lower capacity to methylate arsenic to DMAV. The multivariate-adjusted odds ratios for CAE

of 0, 0.1–15.4, and >15.4 mg/L x year were 1.00, 3.41 (0.74–15.78), and 4.62 (0.96–22.21), respectively (P<0.05, trend test. It was concluded that individuals with a higher arsenic exposure and a lower capacity to methylate inorganic arsenic to DMA^V have a higher risk of developing PVD in the BFD hyperendemic area in Taiwan.

2.5 ARSENIC EXPOSURE PVD (BLACKFOOT DISEASE) AND BLOOD LIPIDS

Tseng CH, Chong CK, Chen CJ and Tai TY (1997)[50] carried out a cross-sectional study to examine whether lipid abnormalities contributed to the endemic peripheral vascular disease (PVD) in villages where arseniasis was hyperendemic in Taiwan. The study was carried out on 533 adults of whom 241 were men and 292 were women. All participants in addition to blood examination for determination of lipid profiles including total cholesterol, triglyceride, high- and low-density lipoprotein cholesterol (HDL-c and LDL-c), apolipoprotein A1 (ApoA1), and apolipoprotein B (ApoB); and assessment for presence or absence of peripheral vascular disease (PVD). For assessment of PVD underwent Doppler ultrasound assisted estimation of systolic blood pressures of bilateral brachial, posterior tibial and dorsal pedal arteries in supine position. And from the systolic blood pressures obtained right and left ABIs were calculated by dividing the higher pressure on the dorsal pedal or posterior tibial arteries on right and left sides respectively by the higher brachial pressure on either side, and any participant having an ABI of less than 0.90 was diagnosed as having PVD. In the study long-term arsenic exposure indices including cumulative arsenic exposure in mg/L-years, duration of drinking artesian well water in years, and duration of living in arsenicosis-hyperendemic villages in years were calculated from detailed history obtained through standardized interviews based on a structured questionnaire and arsenic concentration in well water. Possible confounders including age, sex, body mass index, cigarette smoking, and disease status of diabetes mellitus and hypertension were considered in the analyses. 448947 Among them, 63 had PVD based on an ankle-brachial index < 0.90. None of the lipid profiles (total cholesterol, triglyceride, HDL-c, LDL-c and low-density lipoprotein cholesterol, ApoA1, and ApoB) differed significantly between the presence and absence of PVD. The odds ratios for PVD did not differ among different quintiles of lipid profiles with the lowest quintile as the referent. However, a significant dose-response relation was

found between the long-term arsenic exposure indices and PVD. The multivariate-adjusted odds ratios for cumulative arsenic exposure of 0.1~19.9 and ≥20 mg/L-years were 2.77 and 4.68, respectively, compared with the unexposed. These results suggest that the PVD in arsenicosis hyperendemic villages correlates with ingested inorganic arsenic and not with the lipid profiles.

Tseng WP, Chen WY, Sung JL, et al. (1961)[271] in a study titled 'A clinical study of blackfoot disease-an endemic peripheral vascular disease in Taiwan' cholesterol levels in 41 BFD patients hospitalized for treatment. Among them 32 had normal cholesterol concentration (140 mg/dL to 240 mg/dL) only 2 had serum had serum cholesterol above 240 mg/dL and the remaining 7 patients showed slight decreases of cholesterol (between 100 and 140 mg/dL).

Lee FN, Chen CS, Chen WY. (1983)[280] in a study involving 49 hospitalized full blown BFD cases without diabetes, hypertension and other systemic diseases and 36 normal controls measured blood lipid concentrations (serum cholesterol, triglyceride, and HDL cholesterol). The authors reported only the mean values of total cholesterol, triglyceride, and HDL cholesterol. In the BFD patients and control group, the mean values of the lipid measurements were within normal ranges. The mean values for the BFD patients and the controls, respectively were 185.22±42.55 and 227.44±45.21 mg/dL ($p<0.001$) for cholesterol; 112.22±38.43 and 91.78±34.21 mg/dL ($p<0.01$) for triglyceride; and 41.19±11.52 and 56.58±12.21 mg/dL ($p<0.001$) for HDL cholesterol. Though the differences in blood lipids between the groups were statistically significant the mean values of the lipid measurements were within the normal range.

2.6 EXPOSURE ASSESSMENTS IN ARSENIC EXPOSURE RELATED HEALTH EFFECT STUDIES

When focusing on arsenic exposure related health effects after oral exposure and absorption via alimentary tract, all consumed arsenic needs to be considered to define exposure accurately. Arsenic in the drinking water is most often determined to be in the inorganic form. Besides in drinking water some amount of arsenic could occur in crops, harvested in the contaminated area especially when contaminated water is used for the purpose of irrigation. Significant intake of arsenic through foods cooked using

contaminated water or foodstuffs made from livestock consuming contaminated drinking water can occur. However, most studies reporting on areas of endemic arsenic toxicity usually only drinking water had been considered possibly because of the fact that accurate estimation of arsenic in foodstuffs was difficult and that the amount of arsenic from food was typically much less than that from drinking water[45,50,63,64,241,275,276,281-283]. In the case of large amount of intake of seaweed dietary iAs from food could be considered in the total iAs dose because seaweed is relatively rich in iAs besides arsenosugars[284]. On the other hand, relatively arsenic rich seafood other than seaweed is considered to be of less concern because arsenobetaine is the main arsenic species in seafood and less toxic than iAs[285]. Two major approaches have been used for evaluation of arsenic exposure. Each method has advantages and disadvantages. Thus, it is necessary to observe the situation and to employ the optimum index based on the target symptoms or the health effects.

Concentration of arsenic in drinking water as an index of exposure

Some studies only evaluated the arsenic concentration in the drinking water locally without considering individual consumption volume. Subjects that show large difference from the average consumption of drinking water may vary widely from this estimated individual arsenic exposure. Furthermore, using recently measured drinking water arsenic levels reflects only the present exposure, which may be reasonable for assessing short-term effects, but the dose do not establish exposure levels over a long duration. If the concentration of arsenic in drinking water was stable for a long period, this index of exposure may have some correlation with arsenic related health effects after chronic exposure. When subjects obtained their drinking water form same well continuously throughout their lives, the mean concentration of arsenic in drinking water can be used instead of a time-weighted average, because both indexes result largely in the same theoretical value. Dose in this case is usually expressed as ppm or mg/l or ppb of arsenic in the drinking water.

Daily burden of arsenic from drinking water

Improved index of arsenic exposure is obtained by considering individual daily water consumption of arsenic contaminated drinking water. Daily burden of arsenic is thus calculated by multiplying arsenic concentration of drinking water by the daily intake

volume. Daily consumption can be estimated from self-reporting through interview or questionnaire. Daily individual water consumption is definitely influenced by other factors, for example, weather (air temperature and humidity) and labor intensity, in addition to body size in subjects. Thus, in the existing cases of influencing factors to daily water consumption, daily burden of arsenic from drinking water is better index for estimation of arsenic exposure. Dose is usually expressed as mg/day or mg/kg body weight/day.

Average As exposure

The average arsenic exposure index has an advantage for assessing the relationship between exposure and chronic adverse health effects appearing after long exposure periods. The average arsenic concentration in the drinking water, which has been continuously consumed for a long duration, is calculated in a time-weighted manner by using measured arsenic water concentration multiplied by duration of consumption then divided by total duration. Information on individual water consumption history, including drinking water source, has to be collected by interview or questionnaire, potentially introducing some recall bias. Water samples from all sources used during whole observed period have to be determined for arsenic concentrations. Observation period should be dictated according to end-point of the health effects. Diseases such as cancers would need a long observation period, and the cumulative arsenic exposure for long period should be considered. In this index, the concentration of arsenic in the current drinking water can be substituted for water consumed in the past, assuming that arsenic concentration has been stable, although it is known that there can be seasonal or yearly fluctuations in drinking water arsenic concentration. Dose is usually expressed as ppm or mg/l.

Cumulative As exposure

Cumulative arsenic exposure is calculated as the sum of multiplying arsenic concentration of the drinking water by the duration of consumption and usually expressed in units of (ppm year) or (mg year)/l. In subjects, where drinking water level of arsenic has large variations or where has been a long period of little arsenic exposure years, the index of cumulative arsenic exposure may be suitable. The two parameters concerning the exposure, namely concentration and duration, should be applied appropriately according to the target adverse health effects. Cumulative arsenic exposure index is

suitable for evaluation of dose–response relationships of the adverse health effects appearing after a long period of arsenic exposure.

Biological monitoring as an index of Arsenic exposure

Biomarkers used for monitoring of arsenic exposure have been established and applied widely to estimate individual exposure of workers in occupational health management activities poisoning[286-288]. The amounts of arsenic measured in samples from individual subjects reflect relatively recent arsenic exposure. Knowledge of the chemical form of arsenic is important for biological exposure monitoring. Arsenic in the drinking water, such as from wells, is mostly trivalent or pentavalent iAs. Inorganic arsenic is metabolized by a two-step methylation process in humans, and the total amount of iAs, monomethylarsonic acid (MMA), and imethylarsinic acid (DMA) in the sample should be used as the biomarker of arsenic exposure.

Concentration of arsenic in voided urine

Urine samples are commonly used for evaluation of arsenic exposure levels, owing the ease of sampling and absence of any painful procedure. Calderon et al. (1999)[288] compared arsenic concentrations in spot urine samples and collected urine samples over a 24-h period and found only a small difference between them. These authors also showed minimum fluctuations of urinary arsenic concentration from day to day[288]. Most researchers prefer to use spot urine samples adjusted with creatinine for an arsenic exposure index, instead of the more troublesome 24-h urine collection sampling.

Amount of arsenic in the blood

Peripheral blood samples have also been used for the evaluation of arsenic exposure. Blood samples are obtained by venipuncture but difficulties remain in using this method for mass screening. Both of blood and urine samples reflect individual arsenic intake and are relatively free from outer contamination.

Amount of arsenic in hair

Hair samples are used for a biomarker of arsenic exposure, because iAs and DMA are deposited at hair root and move into hair shafts. The amount of arsenic in longitudinal segments of hair shafts reflects the past arsenic burden at the time when hair was formed. Thus, the total amount of arsenic in a hair sample is a biomarker of the average arsenic exposure over a significant duration of time. Possibilities of external contamination by

washing hair using contaminated water, sweating, or fixation of dust are an issue, because it is not so easy to remove externally deposited arsenic selectively without losing internal arsenic.

Amount of arsenic in nails

Nails of fingers or toes are also used as a biomarker of the average rate of arsenic exposure during a period of time similar to hair samples. Clipped samples of nail reflect arsenic burden from about 3 (finger) or 6 (toe) months ago, since arsenic is deposited by binding to sulfhydryl groups of keratin and then shifts to the tips of the nail as it grows. With nail samples, there are also possibilities of external contamination. Karagas et al. (2002)[289] have discussed the advantages of toenail arsenic concentrations as a biomarker for evaluation of arsenic exposure.

Table No- 13: Parameter of arsenic exposure used in studies relating to dose response relationships between arsenic exposure and health effects

Lesion	Parameter of arsenic exposure	Exposure	Reference
Abnormal pigmentation	mean value of arsenic in hair	mg As/100 g	Borgono et al., 1977[241]
Darkening of skin	Arsenic conc. of drinking water	mg/l	Valentine et al., 1992[281]
Keratosis Hyperpigmentation	Arsenic conc. of drinking water	mg/l	Mazumder et al. 1998[282]
Skin signs	cumulative arsenic exposure	µg/L-years	Haque R et al.[290]
	Arsenic conc. of drinking water mg/L	%	Tsuda et al., 1995[283] Tondel et al., 1999[63]
	% of wells detected (Arsenic >0.05mg/l)	%	Guo et al., 2001[291]
Skin Malignancy	Arsenic conc. of drinking water	mg/l	Wu MM, et al. 1989[275].
Bladder	Total cumulative As dose	mg/l–years	Bates MM, et al., 1995[292]
Lipid profile & PVD	cumulative arsenic exposure	mg/L-years	Tseng CH, et al. 1997[50]
Blackfoot disease	Urinary arsenic	Percentile	Lin and Yang (1988)[276]
Peripheral vascular disease	cumulative arsenic exposure	mg/L-years	Tseng CH, et al. (1996)[45]
Hypertension	cumulative arsenic exposure	mg/L-years	Rahman M, et al. (1999)[64]

Optimal application of indexes for individual exposure

The most desirable indexes for evaluating arsenic exposure for a long period may be obtained by multiplication of the biomarkers of arsenic exposure and exposure durations

in each period. But in cross-sectional study design, such as those commonly used in field research, it is impossible to collect past biological samples to use for biomarker analysis. Thus, a compromise can be introduced for the indexes for arsenic exposure of individual subjects. For the subjects who are living in an area with only small variation of arsenic concentration in the drinking water from all sources, indexes depending on the concentration of arsenic in drinking water or those depending biomarkers could be used with generally equal confidence. On the other hand, for the subjects who are living in an area with large variations of arsenic concentration in the drinking water from all sources, the average or cumulative arsenic exposure, calculated from actual arsenic concentration in drinking water, is the more suitable indexes to apply. Clearly, it is important to select a suitable index of arsenic exposure for the objective assessments of adverse health effects according to the variation of arsenic concentration between each water source and drinking behavior or history of subjects in the target research field. Several reports have discuss the relative risk or prevalence rate of chronic health effects in arsenic exposed populations, and a far fewer papers attempted to define dose–response relationships between arsenic exposure via the drinking water and chronic toxicity because of the difficulty in estimation of individual exposure.

2.7.0 ARSENIC AND ATHEROSCLEROSIS: THE LINK

Atherosclerosis—pathogenic mechanisms

Blood vessel wall is normally composed of an endothelial cell lining on a medial layer of vascular smooth muscle cells and enwrapped by an adventitial layer of connective tissue. And atherosclerosis is a multifactorial pathophysiological process of the arterial vasculature, characterized with progression from inflammation and smooth muscle cell proliferation to late stage of thrombotic and fibrotic obliterations of the vessels. The endothelial cells provide the transduction of signals in the microenvironment between the blood and vessel wall which orchestrate the homeostatic balance of the vessels by production of mediators regulating vascular tone, coagulation status, cell death, and inflammatory cell trafficking. Disruption or activation of endothelial cells leads to series of events including vasoconstriction, increased adhesiveness resulting in inflammatory cell infiltration and platelet-thrombus formation[293-295]. Activation of the endothelium appears to be the key event in the initiation of atherosclerosis [296].

And endothelial cell dysfunction is associated with alteration of the cellular redox state. Oxidative modification of low-density lipoprotein (LDL) particles is a dominant hypothesis of atherogenesis[297-300]. Oxidized LDL particles are readily taken by macrophage scavenger receptors, leading to ''foam cell'' formation (macrophage loading with lipids), an obligatory step in the development of atheroma. Bioactive lipids derived from LDL oxidation are also capable of modulation of intracellular signal transduction and expression of genes coding inflammatory mediators and adhesion molecules [301-303]. Furthermore reactive oxygen species (ROS) can directly function as signaling molecules that can lead the induction of activity of nuclear transcription factors (such as NF-kB and AP-1)[303-306]. Increased activity of these transcription factors is associated with up-regulation of vascular adhesion molecules (VCAM)-1 and chemokines including monocyte chemoattractant protein (MCP)-1 and interleukin (IL)-8 in the endothelium[307-309]. Chronic inflammation has been demonstrated histopathologically as an accompanying event in atheroma formation and may be an important modifying factor of atherosclerosis progression [310].

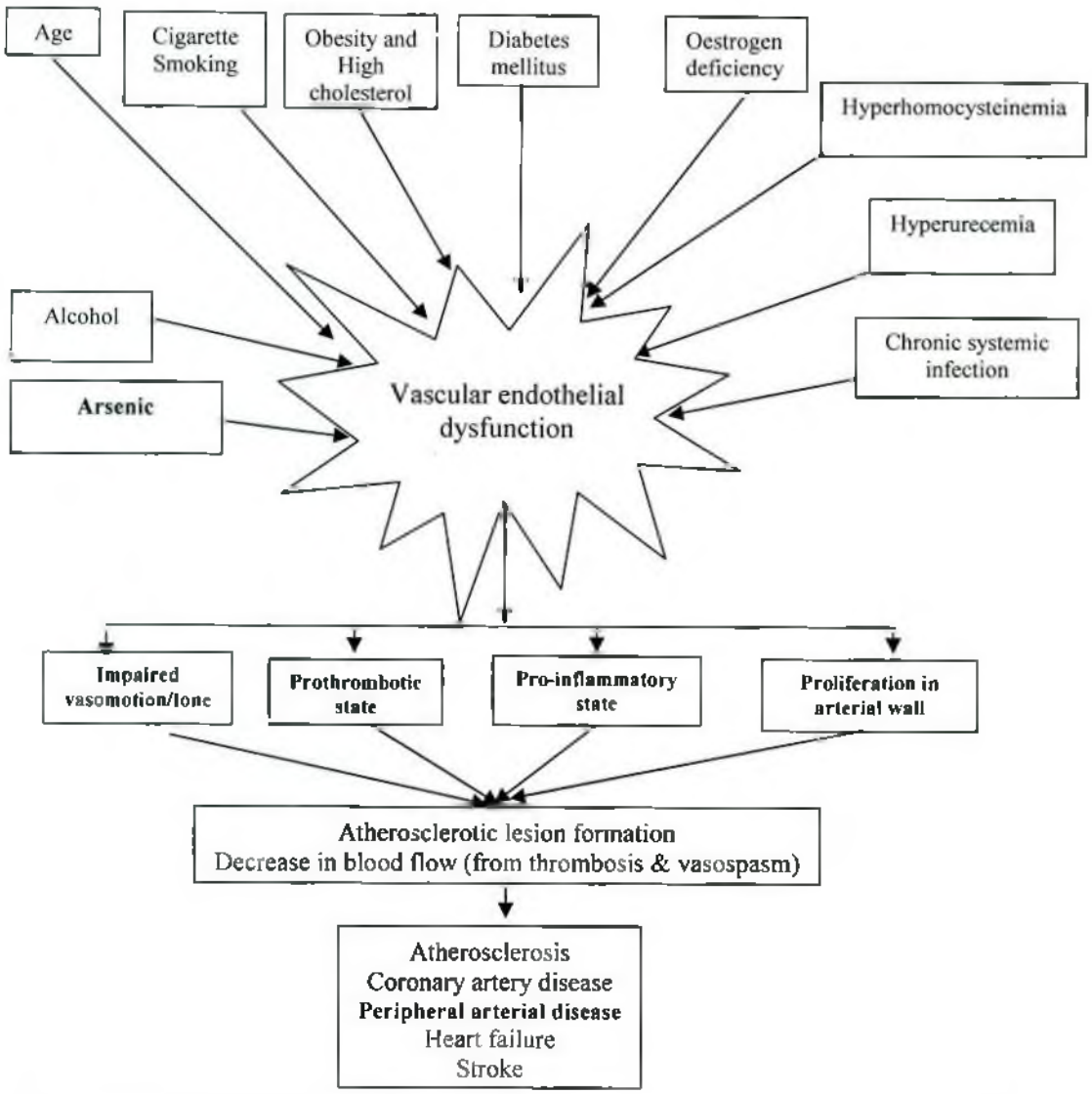


Figure no- 19: Risk factors associated in the pathogenesis of vascular endothelial[297]

Endothelial dysfunction and atherogenesis have also been strongly related to an impaired nitric oxide (NO) homeostasis in the vessel[294,311]. Nitric oxide (NO) produced in endothelial cells is involved in the regulation of blood pressure, inhibition of the adhesion of leukocytes to the endothelium, interactions between platelets and the vessel wall, and proliferation and migration of vascular smooth muscle cells [312,313]. NO plays multiple physiological roles in vascular wall including endothelium-mediated vasodilatation, inhibition of platelet activation and smooth muscle cell migration and proliferation, and suppression of the pro-inflammatory mediators through NF-kB inactivation. Attenuation in NO bioavailability may be caused by inhibition of the endothelial NO synthase (eNOS) expression, a lack of substrate or cofactors for eNOS, alterations of cellular

signaling such that eNOS is not appropriately activated, and, finally, NO inactivation through interaction with ROS such as superoxide anion [293,314,315]. Many risk factors, including cigarette smoking, hypertension, diabetes mellitus, hypercholesterolemia, herpes viruses, and Chlamydia pneumoniae, can induce atherogenesis by modulation of inflammatory potential, oxidative stress, or NO perturbations in the endothelium[316,317].

Arsenic-induced molecular and cellular events related to atherogenesis

In vitro studies with endothelial cell cultures suggested that arsenic can cause cellular redox modulation, transcription factor activation, and gene expression relevant to endothelial dysfunction[233, 234, 236, 237, 240,297,318-341]. Some of the characteristics of arsenic can explain its effect of atherogenicity is shown in table no-14.

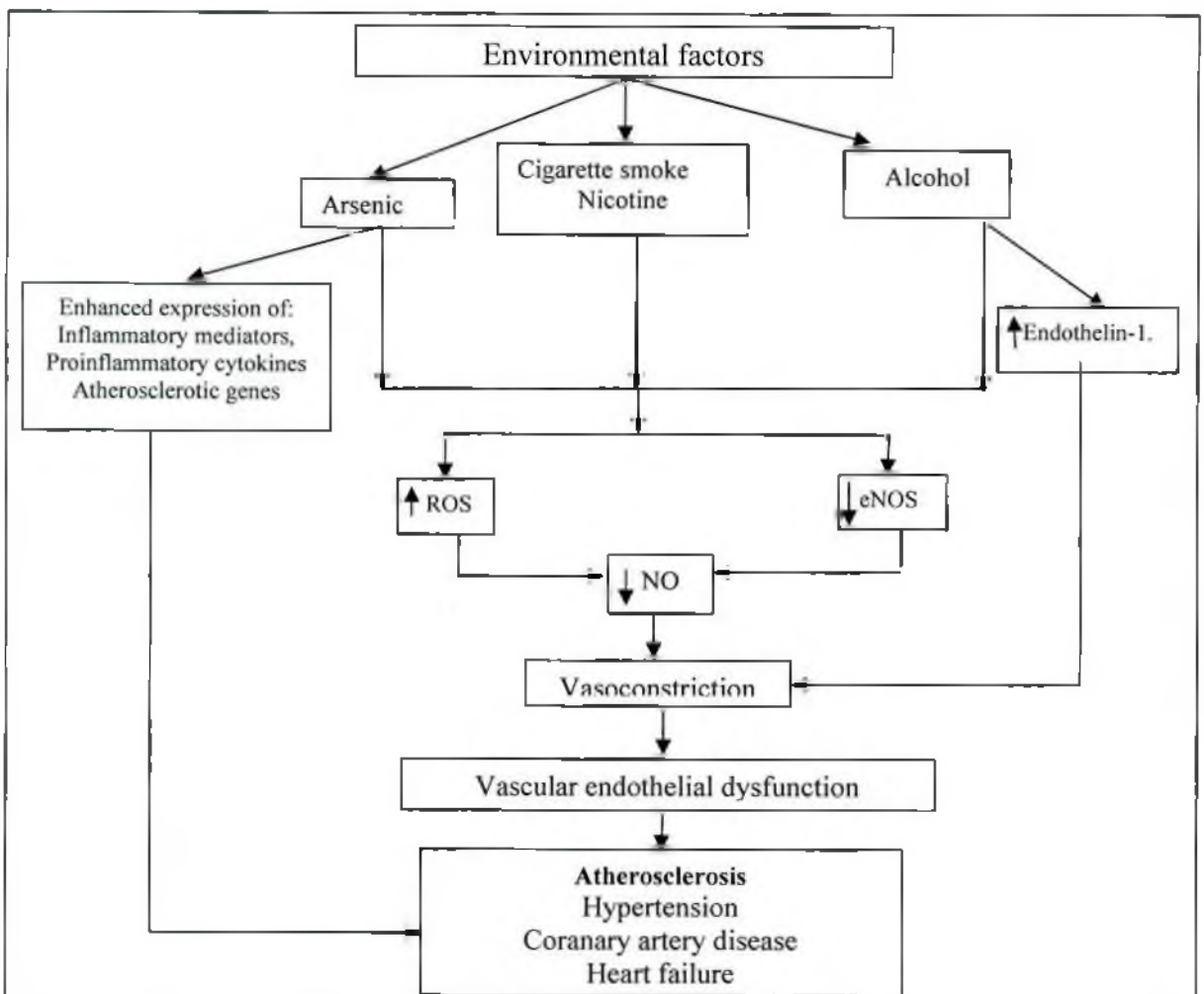


Figure No-20: Mechanisms involved in environmental factors-induced vascular endothelial dysfunction. ROS indicates reactive oxygen species; NO indicates nitric oxide; eNOS indicates endotheial nitric oxide synthase; ACE indicates angiotensin converting enzyme; VED indicates vascular endotheial dysfunction[297].

Bunderson M et al (2004)[318] demonstrated induction of 5-lipoxygenase (5-LO) accompanied by significant increase in leukotriene E4 (LTE4) and prostacyclin (PGI2) in the serum of arsenic-treated mice. 5-LO and other LO enzymes have been linked to atherosclerosis[296,319]. Upregulation of the inflammatory mediator cyclooxygenase-2 (COX-2) accompanied by increase in the generation of prostaglandin E2 in bovine aortic endothelial (BAE) cells exposed to arsenite was also been demonstrated [320]. Induction of such inflammatory mediators is likely to exacerbate the inflammatory state typical of atherosclerosis.

Table no-14: Pathogenetic mechanisms of atherogenicity induced by arsenic[233, 234, 236, 237, 240,297,320-341].

Endothelial cell damage
Endothelial cell dysfunction
Increased inflammatory activity
Increased coagulability
Decreased fibrinolysis
Interference with functions of DNA, RNA, and proteins
Increased oxidative stress with impaired nitric oxide balance
Smooth muscle cell proliferation
Induction of apoptosis
Interaction with other trace elements
Association with hypertension and diabetes mellitus.

It has been demonstrated that exposure of endothelial cells to arsenite induces NF-kB activation and DNA synthesis through reactive oxygen[136,333] as well as decreases Fas ligand expression through oxidant formation[238] arsenite initiates vascular dysfunction by activating oxidant-sensitive endothelial cell signaling. Recently, it has been reported that arsenic increases the expression of cyclooxygenase-2 in bovine aortic endothelial cells as well as human umbilical vein endothelial cells (HUVEC) through peroxynitrite formation and NF-kB activation, respectively [320,323].

It has also been shown that arsenic induces an increase of intracellular oxidized glutathione (GSSG) in endothelial cells which may lead to oxidative stress[236,329,342,343]. It has been demonstrated an uptake of arsenic into endothelial cells and concomitant induction of antioxidative enzymes, including heme oxygenase-1, thioredoxin perooxidase-2, NADPH dehydrogenase, and glutathione S-transferase P subunit, which suggests arsenic-induced oxidative stress[330,236]. Other investigators have also demonstrated that arsenic disrupts endothelial cell proliferation [233, 243,344].

iAsIII has been found to modulate cytokine secretion and increase the production of many cytokines including the tumor necrosis factor-α (TNFα), the proinflammatory cytokine interleukin 1a (IL-1a)[345], the neurotrophic cytokine IL-6, the neutrophil chemotactic cytokine IL-8 [346,347] as well as cellular growth factors such as the transforming growth factor-a (TGFa) and the granulocyte macrophage-colony stimulating factor (GM-CSF)[348,349]. Exposure of human aortic endothelial cells (HAEC) to arsenic has been found to result in expression of genes coding for inflammatory mediators including IL-8[322]. Atherogenesis requires local chemokine production for regulating migration and activation of leucocytes. Classically, IL-8 is recognized as a major neutrophil chemotactic factor, but consistent observations of increased IL-8 expression without neuthrophil involvement in atherosclerotic lesions suggested alternative roles of IL-8 in atherosclerosis[350]. Consistently, it has been demonstrated that IL-8 is an angiogenic factor[351] and chemotactic for vascular smooth muscle cells[352,353], T-cells[353], and monocytes[355]. The expression of IL-8 by arsenic was accompanied with activation of transcription factors, including NF-kB and AP-1. Injury to the endothelium and local chemokine production are thus likely to promote accumulation of oxidized low-density lipoproteins (oxLDLs) and monocytes/macrophages in the neointima of blood vessels[319], thus leading to formation of foam cells ultimately formation of an atheroma. Pereira FE et al (2007)[327] have demonstrated that As(III)-induces activation of PKCα causes PYof β-catenin and formation of stress fibers, which in association with the contractile force generated by stress fibers, could result in gap formation and increased endothelial permeability. The intercellular gaps and increased permeability caused by arsenic could potentially accelerate/enhance influx of oxLDLs and monocytes/macrophages across the endothelium and contribute significantly to the progression of atherosclerosis.

Previous studies also demonstrated that arsenic is a potent activator of AP-1 and NF-kB DNA binding activity in different type of cells including porcine aortic endothelial cells, keratinocytes, and urinary bladder cells[332,356,357]. AP-1 and NF-kB can regulate inflammatory gene expression, for example, the IL-8 gene promoter region contains multiple binding sites for these transcription factors [358,359] and the transcription factors are subject to redox-dependent regulation[360,361]. Consistent with the ''lipoprotein oxidative-modification hypothesis'' of atherosclerosis, free transition metal ions, such as copper,

have been shown to stimulate the lipoprotein oxidation by endothelial cells in vitro[362]. Although arsenic is not a transition metal, it has been shown to posses an oxidative potential. However, we were unable to demonstrate that arsenic, in contrast to copper or hydrogen peroxide, induces oxidation of LDL in human aortic endothelial cells[322].

Accumulating evidence demonstrates that arsenic exposure modulates the coagulation status. In this respect, arsenic has been shown to enhance the aggregation activities of platelets[324].

Exposure of human microvascular endothelial cells to arsenic in vitro resulted in a decrease of tissue-type plasminogen activator (t-PA) and an increase of plasminogen activator inhibitor type-1 (PAI-1) expression as well as reduced fibrinolysis [325,363]. Increased coagulating and lowered fibrinolytic activities contribute to endothelial dysfunction and atherogenesis through induction of thrombotic events in the circulation.

Arsenite exposure has been recently related to an impaired NO homeostasis [329,333,334]. Arsenic inhibited acetylcholine induced relaxation of aortic rings and reduced the levels of guanosine 3,5-cyclic monophosphate (cGMP), a surrogate for NO[321,364]. These effects were consistent with concentration-dependent eNOS inhibition in endothelial cells. Arsenic-mediated impaired vasomotor tone, as a result of reduced NO bioavailability, may contribute to arsenic-related atherosclerosis and hypertension.

All of the atherosclerosis-related effects induced by arsenic, including transcription factor activation, gene expression, or an impaired NO homeostasis, might be mediated indirectly by induction of oxidative stress (as discussed above) or directly through arsenic reactivity to vicinal sulfhydryl groups[329]. Macromolecules involved in cell signaling, such as receptors, integrins, or protein phosphatases contain high numbers of vicinal sulfhydryls and are capable of reacting with arsenic[236,237,326-328].

Pysher MD et al (2008)[353] have demonstrated that arsenic induces alterations in the co-association and activity of Focal Adhesion (FA) proteins in primary VSMCs. And these alterations results in a sustained increase in FAK–src association and activation, as well as formation of unique signaling complexes. The effects of these alterations were manifested as chronic stimulation of PAK1 and downstream ERK and JNK MAPK pathways resulting in VSMC growth, proliferation, and migration. Thus, the data presented here suggest a potential role for arsenic, via activation of these same pathways,

as a mediator of VSMC behavior. Thus it appears that arsenic induces pathophysiological events relevant with atherogenic potential including increased oxidative stress, inflammatory and coagulation activity of endothelium and impaired vascular nitric oxide homeostasis (Fig no- 19).

2.8.0 ARSENIC EXPOSURE AND OXIDATIVE STRESS: EVIDENCE FROM HUMAN STUDIES.

Pi JB et al 2000[365] in a study carried out in Wuyuan, the southwestern area of Inner Mongolia, to determine relationship between NO synthesis and chronic arsenic poisoning in humans. The subjects were 33 habitants who continued to drink well water containing high concentrations of inorganic arsenic (mean value 0.41 μg/ml) for about 18 years in Inner Mongolia, China, and 10 other people who lived in this area but exposed to minimal concentrations of arsenic (mean value 0.02 μg/ml) were employed as controls. Serum levels of nitrite/nitrate, the stable metabolites of endogenous NO, were almost 50% lower in patients chronically exposed to elevated arsenic in the drinking water than in control subjects Mean blood concentration of total arsenic was six times higher in exposed subjects than controls; 42.1 vs. 7.3 ng/ml, $p<0.001$. Mean serum concentration of nitrite/nitrate, stable metabolites of endogenous NO, was lower in arsenic-exposed subjects than in controls: 24.7 vs. 51.6μM, $p<0.001$. In total samples, an inverse correlation with serum nitrite/nitrate levels was strong for blood inorganic arsenic ($r = -0.52$, $p<0.001$) and less strong for its metabolites, monomethyl arsenic ($r = -0.45$, $p<0.005$) and dimethyl arsenic ($r = -0.37$, $p<0.05$). Furthermore, serum nitrite/nitrate concentration was significantly correlated with nonprotein sulfhydryl level in whole blood ($r = -0.58$, $p<0.001$). These results suggest that long-term exposure to arsenic by drinking well water possibly reduces NO production in endothelial cells, resulting in a decrease in reduced nitrite/nitrate concentrations. Peripheral vascular disorders caused by arsenic may be attributable in part to impairment of NO production in vivo.

Wu et al. (2001)[366] in an attempt to investigate the effect of arsenic exposure on oxidative stress in humans, conducted a population study to determine the relationships of blood arsenic to reactive oxidants and antioxidant capacity at the individual level. In the study

64 subjects aged 42–75 years were recruited from among residents of the Lanyang Basin on the northeast coast of Taiwan, where arsenic content in well water varies from 0 to ≥3,000 μg/L. A chemiluminescence method, with lucigenin as an amplifier for measuring superoxide to measure the plasma level of reactive oxidants, and the azino-diethyl-benzthiazoline sulphate method to determine the antioxidant capacity level in plasma of each study subject were used. Arsenic concentration in whole blood was determined by hydride formation with an atomic absorption spectrophotometer. The average arsenic concentration in whole blood of study subjects was 9.60 ± 9.96 μg/L (± SD) with a range from 0 to 46.50 μg/L. The level of arsenic concentration in whole blood of study subjects showed a positive association with the level of reactive oxidants in plasma (r = +0.41, p=0.001) and an inverse relationship with the level of plasma antioxidant capacity (r = –0.30, p = 0.014). However, no significant association (p =0.266) between levels of plasma reactive oxidants and antioxidant capacity was found. The results also showed that the lower the primary arsenic methylation capability, the lower the level of plasma antioxidant capacity (p = 0.029). These results suggested that ingestion of arsenic-contaminated well water may cause deleterious effects by increasing the level of reactive oxidants and decreasing the level of antioxidant capacity in plasma of individuals. Persistent oxidative stress in peripheral blood may be a mechanism underlying the carcinogenesis and atherosclerosis induced by long-term arsenic exposure.

Pi JB et al 2002[367] in a cross-sectional study in Wuyuan, Inner Mongolia, China, to explore the relationship between chronic arsenic exposure from drinking water and oxidative stress in humans. Thirty-three inhabitants who had been drinking tube-well water with high concentrations of inorganic arsenic (mean value = 0.41 mg/L) for about 18 years constituted the high-exposure group, and 10 residents who lived nearby but were exposed to much lower concentrations of arsenic in their drinking water (mean value = 0.02 mg/L) were selected as the low-exposure comparison group. Results of the present study indicated that although the activity for superoxide dismutase (SOD) in blood did not differ significantly between the two groups, the mean serum level of lipid peroxides (LPO) was significantly higher among the high-exposed compared with the low-exposed group. Elevated serum LPO concentrations were correlated with blood levels of inorganic

arsenic and its methylated metabolites. In addition, they showed an inverse correlation with nonprotein sulfhydryl (NPSH) levels in whole blood. The subjects in the high–arsenic-exposure group had mean blood NPSH levels 57.6% lower than those in the low-exposure group. Blood NPSH levels were inversely correlated with the concentrations of inorganic arsenic and its methylated metabolites in blood and with the ratio of monomethylarsenic to inorganic arsenic. These results provide evidence that chronic exposure to arsenic from drinking water in humans results in induction of oxidative stress, as indicated by the reduction in NPSH and the increase in LPO.

2.9.0 ARSENIC AND ATHEROSCLEROSIS EVIDENCE FROM ANIMAL MODELS

The wild-type C57BL/6 mice are resistant to atherosclerosis but atheroma formation can be triggered by long-term (more than 24 weeks) high-fat diet[368]. Progress in understanding the risk factors leading to atherosclerosis have resulted from development of specific genetic mouse models that make the mouse from very resistant to highly susceptible of atherosclerosis. One of these models is deficiency of the gene coding apolipoprotein E (ApoE), one of several lipoprotein transfer genes[369,370]. The primary function of ApoE protein is mediation of receptor-dependent lipoprotein removal from the blood. The ApoE-deficient mouse develops massive fibroproliferative atherosclerosis when fed a low-fat and low-cholesterol chow diet[368]. The lesions are dispersed throughout the arterial tree forming at the base of the aorta, in the proximal coronary arteries, and along the entire length of the aorta, with predisposition at the branch points of major vessels leaving the aorta. In terms of anatomical localization and histopathological characteristics of the plaques, this model more closely resembles human atherosclerosis compared to the high-fat diet-induced mouse (wild type) atherosclerosis model. Arsenic exposure, through drinking water, was found to increase atheroma formation in ApoE-/- mice in parallel with increasing levels of arsenic in the vessel wall[322].

A recent study treating ApoE−/−/LDLr−/− mice with 133 μM (10 ppm) sodium arsenite in drinking water for 18 weeks has successfully induced a significant increase in

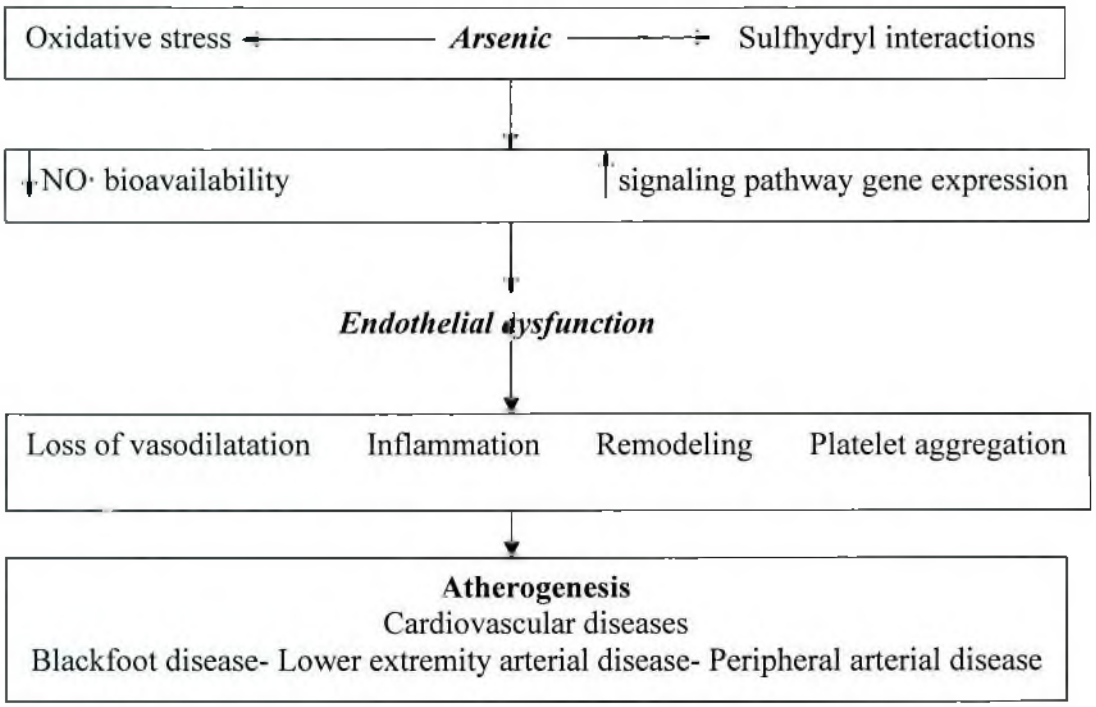


Figure No-21: Schematic representation showing possible mechanisms involved in arsenic-related atherogenesis and cardiovascular diseases.

atherosclerotic plaques in the innominate artery while compared to controls[320]. This animal model provided evidence for biological plausibility of arsenic-induced atherosclerosis observed in humans in epidemiologic studies.

2.10.0 THE HUMIC ACID ISSUE

In addition to arsenic, several compounds, like ergotamine, organic chlorides, and fluorescent substances had also been found in this artesian well water. These fluorescent compounds known as humic acids are a group of polymers with high molecular weights that results from the decomposition of organic matter, particularly dead plants. The humic acids which are fluorescent compounds are widely spread contaminants of water harnessed from groundwater aquifers had also been suggested as a cause of BFD[371,372,373]. Lu et. al.[374] in an experimental study on 16 mice that did not include controls, demonstrated that after intraperitoneal injection of dissolved crystallized fluorescent compounds 50% developed gangrene of the tails and feet but the pathological changes was not similar to that as observed in BFD. In a large series of 300 mice exposed to humic acids in their drinking water for 24 months, no gangrenous changes were

reported[375]. The fluorescent compounds seem to induce acute thrombosis via an action on blood coagulation[376], which is pathologically quite different from BFD. However, there has been no epidemiologic evidence presented to date to show a correlation between exposure to humic substances and development of PVD and the association between humic substances and BFD might be due to the confounding effect of the strong association between BFD and arsenic exposure[45,270]. Recent studies have demonstrated that humic acids have also been shown to induce the generation of nitric oxide in human umbilical vein endothelial cells leading to cell injury[377], induce apoptosis[378], and increase the adhesibility of neutrophils[379]. However, all of these effects can also be demonstrated with arsenic exposure. Humic acids have not been shown to cause the dermatological or neurological defects, which are typically seen in humans[273,380] or animals[381,382] exposed to arsenic.

Although arsenic is the most probable cause of BFD, the potential role of other factors such as the humic acids cannot be completely excluded. The effects of arsenic and humic acids may not be mutually exclusive and their interaction on the promotion of BFD remains to be elucided[270].

CHAPTER III

MATERIALS AND METHODS

3.1 STUDY DESIGN

A cross-sectional comparative study was carried out to assess if exposure to excess arsenic through drinking water possess any risk difference for LEAD in Bangladesh. The study participants were included the following groups:

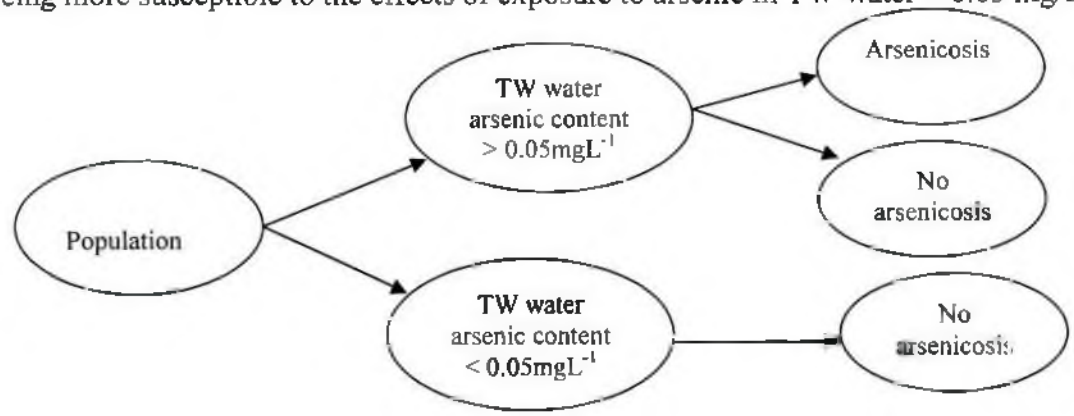
a. ***Symptomatic exposure group (arsenicosis)***: Individuals having the signs of arsenicosis (bilateral palmoplantar keratosis ± melanosis) and evidence of having consumed (is consuming) tubewell water that contained arsenic in excess of 0.05 mg/L.

b. ***Asymptomatic exposure group (non arsenicosis)***: Individuals having evidence of having consumed (is consuming) tubewell water that contained arsenic in excess of 0.05 mg/L but not having any of the signs of arsenicosis (bilateral palmoplantar keratosis ± melanosis on the trunk).

c. ***Reference group***: Individuals having evidence of not having consumed tubewell water whose arsenic content was in excess of 0.05 mg/L and not having any signs simulating the signs of arsenicosis (bilateral palmoplantar keratosis ± melanosis on the trunk).

3.2 STUDY POPULATION(S)

In the context of arsenic exposure situation in Bangladesh, the human population can be clearly categorized into two broad groups; those consuming tubewell water having arsenic >0.05 mg/L, and those consuming tubewell water whose arsenic content is <0.05 mg/L. Again, the group consuming tubewell water having arsenic >0.05 mg/L can be subdivided into two subcategories; those having the signs of arsenicosis and those not having the signs of arsenicosis. Those having signs of arsenicosis most probably constitutes a especial group in the population in the sense that they could be considered as being more susceptible to the effects of exposure to arsenic in TW water > 0.05 mg/L.



3.3 SAMPLE SIZE

The procedure of sample size calculation for studies about proportions in two groups available in the NCSS/PASS software[383] was used in calculating sample size for this study.

P_1 = anticipated value of the proportion having LEAD amongst the population having exposures to arsenic through tubewell water whose arsenic concentration is <0.05 mgL^{-1} (reference group)= 3%[9,16].

P_2 = anticipated value of the proportion having LEAD amongst the population having exposures to arsenic through tubewell water whose arsenic concentration is > 0.05 mgL^{-1} and having signs of arsenicosis (symptomatic exposure group) = 6%.

Null Hypothesis: $P_1=P_2$ Alternative Hypothesis: $P_1<P_2$.

The sample sizes adequate enough to test the hypotheses at 5% level of significance with 90% & 80% power and using continuity correction turned out to be as follows:

Table no-15: Group sample sizes at 5% level of significance with 90% & 80% power.

Power	P_1	P_2	Allocation Ratio	Odds Ratio	N_1	N_2	Alpha	Beta
0.90028	0.03	0.06	1.00	2.064	861	861	0.0500	0.09972
0.80059	0.03	0.06	1.00	2.064	639	639	0.0500	0.19941

It was found that group sample sizes of 861 and 861 could achieve 90% power in detecting a difference of 0.03 between the null hypothesis that "both group proportions are 0.03" and the alternative hypothesis that "the proportion in group 2 is 0.06" using a one-sided Chi-square test with continuity correction and with a significance level of 0.05. Based on the response rate (86.2%) of a study on diabetes amongst rural population[384] it was assumed that inflation of the sample size by 20% would accommodate for any withdrawal from participation at different stages of data collection and for incompleteness of data if any. Based on the above considerations it was decided to include 1000 participants in each of the groups. As the study has been designed to include 3 independent groups (symptomatic exposure group, asymptomatic exposure group and a reference group) a total of 3000 participants, with equal numbers (1000) in each group was considered to be sufficient enough for the study.

3.4 CRITERIA FOR RECRUITMENT OF PARTICIPANTS FOR DIFFERENT STUDY GROUPS:

Symptomatic exposure group (Group I)

a. Inclusion criteria-

1. Individuals not less than 30 years of age but not more than 60 years.
2. Both sex.
3. Residing in the current residence for at least 10 years and resident of the upazilla since birth.
4. Individuals having the most classical signs of arsenicosis (bilateral palmoplantar keratosis ± melanosis on the trunk).
5. Individuals having evidence of having consumed (is consuming) tubewell water that contained arsenic in excess of 0.05 mg/L.

b. Exclusion criteria-

1. Individuals less than 30 years of age and more than 60.
2. Individuals having been residents of the village for less than 10 years.
3. Individuals not having the classical signs of arsenicosis (bilateral palmoplantar keratosis ± melanosis on the trunk).
4. Females who are pregnant or in postpartum period.
5. Individuals having evidence of having consumed (is consuming) tubewell water whose arsenic content is/was less 0.05 mg/L.
6. Individuals, who are severely anemic, dehydrated, in the state of shock or are severely debilitated.

Asymptomatic exposure group (Group II)

a. Inclusion criteria-

1. Individuals not less than 30 years of age but not more than 60 years.
2. Both sexes.
3. Residing in the current residence for at least 10 years and resident of the upazilla since birth.
4. Individuals not having any of the signs of arsenicosis (bilateral palmoplantar keratosis ± melanosis on the trunk).

5. Individuals having evidence of having consumed (is consuming) tubewell water that contained arsenic in excess of 0.05 mg/L.

b. Exclusion criteria-

1. Individuals less than 30 years age or more than 60 years.
2. Individuals having been residents of the village for less than 10 years.
3. Individuals having the signs of arsenicosis (bilateral palmoplantar keratosis or melanosis on the trunk) or signs simulating the signs of arsenicosis.
4. Females who are pregnant or in postpartum period.
5. Individuals having evidence of having consumed (is consuming) tubewell water whose arsenic content is/was less 0.05 mg/L.
6. Individuals, who are severely anemic, dehydrated, in the state of shock or who are severely debilitated.

C. Reference group (Group III):

a. Inclusion criteria-

1. Individuals not less than 30 years age but not more than 60 years.
2. Both sexes
3. Residing in the current residence for at least 10 years and resident of the upazilla since birth.
4. Individuals not having any of the signs of arsenicosis (bilateral palmoplantar keratosis ± melanosis on the trunk).
5. Individuals having evidence of not having consumed (is consuming) tubewell water that contained arsenic in excess of 0.05 mg/L.

b. Exclusion criteria-

1. Individuals aged less than 30 years or more than 60 years.
2. Individuals having been residents of the village for less than 10 years.
3. Individuals having signs simulating the signs of arsenicosis.
4. Females who are pregnant or in postpartum period.
5. Individuals having evidence of having consumed (is consuming) tubewell water whose arsenic content is/was less 0.05 mg/L.

6. Individuals, who are severely anemic, dehydrated, in the state of shock or who are severely debilitated.

3.5 RESEARCH INSTRUMENTS

The following research instruments were used in study participant recruitment and data collection:

1. Participant Eligibility Screening Forms: These forms were used for determining the eligibility of anticipated participants for inclusion in the study (annex 1-3). The eligibility forms included information on age, gender, residential information and information related to criteria for inclusion and exclusion.
2. Consent form and information sheet: An information sheet was designed stating the objectives of the study; what was expected from the participants; what the participants could expect out of their participation in the study; and of their freedom to withdraw from the study at any point of their participation (annex 4). A consent form was designed to obtain the consent of an eligible participant (annex 5).
3. Household Questionnaire: The household questionnaire was designed so as to elicit sociodemographic information, to assess selected lifestyle information, to assess food consumption frequency, obtain medical history pertaining to LEAD, and information required to undertake arsenic exposure assessment through drinking water (annex -6).
4. Medical Examination Checklist/schedule: A medical examination checklist/schedule was designed for the purpose of general medical history & examination, anthropometrical assessment, blood pressure status assessment, ABI assessment, and assessment diabetic status (annex-7).

3.6 VARIABLES OF THE STUDY (*including operational definitions*)

A. LEAD: Any individual having had amputation following gangrene or having a Doppler assisted ABI of 0.90 or less was considered as having LEAD.

B. ABI: ABIrefers to ratio of systolic pressure obtained at posterior tibial &/or dorsalis pedis arteries to that obtained at the brachial artery[14,21,23-25,29,30,228,229]. All measurements undertaken in supine position after 5 minutes of rest.

C. Sociodemographic variables

1. Age: As stated by the study participant in complete years.
2. Sex
3. Literacy status: academic achievements in terms of level of schooling or degree/diploma obtained.
4. Family income: Sum of earnings of all members of the family of the study participant
5. Household type: The physical characteristics of the floor, walls, & roof were taken into consideration in determining the household type of the participant.
6. Family size: Total number of the family members of the study participants who were resident of the household.
7. Occupation: The occupation that involves most of the time of the respondent was taken as the occupation.

D. Drinking water source (since childhood)

1. Type (tubewell, dugwell, ponds, river, rainwater, etc.)
2. Duration for each source as stated by the participant and information on all sources used and their types of sources

E. Arsenic level in water from source(s) used for collection of drinking water as estimated through hydride generation using atomic fluorescence spectrophotometry (AFS) at NIPSOM-DOEH arsenic lab (annex-7)

F. Cumulative arsenic exposure: sum of the products obtained by multiplying the arsenic concentration of the tubewell water (mg/L) by duration (years) of water consumption from individual tubewell was taken as the cumulative arsenic exposure (mg years/L)[45,50,64,292].

G. Food consumption frequency (one week): Food consumption frequency refers to number of days in the preceding week an individual had consumed the

following items vegetables, meat, fish, eggs, and milk or milk product (dohi).

H. Smoking habit: This refers to current smokers, past smokers and ever smokers.

I. Body mass index (BMI): BMI of each respondent would be obtained by dividing their body weight (in kg) with the squared value of their height (in meter). BMI would be of three categories[385]

   a. Normal $<25kg/m^2$

   b. Overweight $\geq 25kg/m^2$ but $<30kg/m^2$

   c. Obese-BMI $\geq 30kg/m^2$

J. Blood pressure status: Blood pressure status refers to the state of blood pressure obtained over the right brachial artery in sitting posture after 5 minutes rest. It would be of three categories:[386]

   a. Hypertension-Any individual having a systolic blood pressure of >139 mm Hg or a diastolic blood pressure >89 mm Hg, or has previously been diagnosed as having hypertension and taking antihypertensive medications would be considered as having hypertension.

   b. Prehypertension- Any individual having a systolic blood pressure falling in the range of 120-139 mm Hg or a diastolic blood pressure falling in the range of 80-89 mm Hg.

   c. Normotensive- individual having a systolic blood pressure of <120 mm Hg and a diastolic blood pressure <80 mm Hg,

K. Glycaemic state: Glycaemic state refers to the state as dictated by the blood glucose levels estimated from finger prick capillary blood. It would be of two categories[385,387]:

   a. Diabetic- Any individual previously been diagnosed as having diabetes mellitus and are on oral or parenteral hypoglycaemic agent(s); or a fasting capillary blood glucose level of ≥6.1mmol/L (≥110mg/dl); or a fasting capillary blood glucose level of <6.1mmol/L (<110mg/dl) but having a capillary blood glucose level of ≥11.1mmol/L (≥200mg/dl) 2

hour after glucose load was considered as having diabetes mellitus.

b. Normal (euglycaemic)- Any individual not having been previously diagnosed as having diabetes mellitus and are not on oral or parenteral hypoglycaemic agent(s), and having a fasting capillary blood glucose level of <6.1mmol/L (<110mg/dl) and a capillary blood glucose level of <11.1mmol/L (<200mg/dl) 2 hour after glucose load was considered as non-diabetic (normal/euglycaemic).

3.7 SELECTION OF STUDY AREA

Initially from available patient lists available with NIPSOM, Asia Arsenic Network (AAN), WHO, HEED Bangladesh, ICDDR,B and DGHS were examined to identify upazillas from which 200 or more arsenicosis patients had been identified. Arsenicosis patients lists of Chapai Nawabganj Sadar (DGHS & NIPSOM), Mujibnagar (HEED Bangladesh & NIPSOM), Gangni (HEED Bangladesh & NIPSOM), Sharsha (AAN & NIPSOM), Alamdanga (HEED Bangladesh), Bhanga (WHO, NIPSOM, DGHS), Shahrasti (DGHS & ICDDRB), Chatkhil (Dr Momin, Dept of Dermatology, DMCH), Lakhsam (DGHS & ICDDRB), Muradnagar (WHO & DGHS), Sirajdekhan (WHO & DGHS), and Monohordi (DGHS & NIPSOM) upazillas were examined for availability of information sufficient enough so as to be able to trace the enlisted arsenicosis patients. The upazillas were visited and the local health authorities were approached to find out the situation in their respective jurisdiction. The issue of feasibility of carrying out the planned study was discussed and the availability of space and logistics for setting up health camps at each upazilla health complex was explored.

Ultimately Alamdanga of Chuadanga district, Shahrasti of Chandpur district and Lakhsam of Comilla district were selected for recruiting participants of Gr I and Gr II.

As for selection of an area for recruitment of participants for the reference group (Gr III) the BAMWSP database[227] was explored to identify upazillas with no arsenicosis patients and where least numbers of tubewells were arsenic contaminated (arsenic content of water <0.05 mg/L). Sreepur and Kapasia upazillas of Gazipur district and Pakundia

upazilla of Kishorganj district were initially selected. The upazillas were visited and the local health authorities were approached to find out the situation in their respective jurisdiction. The issue of feasibility of carrying out the planned study was discussed and the availability of space and logistics for setting up health camps at each upazilla health complex was explored. Of these upazillas Sreepur and Kapasia upazillas the households were sparsely located and the housing type was very different from that in the areas selected for recruitment for participants for Gr I and Gr II.

Table No-16: List of villages from which study participants were recruited.

Upazilla*	***Villages***		
Alamdanga (Chuadanga)	Notidanga	Domdoma	Takpara
	Damosh	Bengari	Boaleshpur
	Betabari	Gorchapra	Krishnopur
Shahrasti (Chandpur)	Bishara	Malra	Rayosree
	Debkoara	Malora	Sreepur
	Kazirkup	Noagao	Voldighi
	Kazinagar	Padua	
Lahksam (Comilla)	Pashapur	Bamodda,	Aushtola
	Salehpur	Dhankonia	Chonagao
	Dumoria	Changachal	Ronachu
	Singjoir	Shahpukoria	Noagao
	Hamirabad	Eruaine	Monohorpur
	Vakoidda	Auspara	Kandirpar
	Paschimgaon	Dimatoi	
Pakundia (Kishorganj)	Char Pakundia	Kurotola	Maddha Pakundia
	Lokia	Boratia	Chalia ghoup
	Diapara	Dordora	Morura
	Happania	Paiklokia	

* Names of districts in parentheses

Pakundia upazilla of Kishorganj district was selected for recruiting participants of Gr III. Based on further examination of the details of the BAMWSP screening reports 11 villages of Pakundia upazilla were finalized for recruitment participants of Gr III.

3.8 PARTICIPANT RECRUITMENT PROCESS

Initially the households of arsenicosis patients selected from available patient lists were visited and their eligibility to be study participants was assessed using the pretested eligibility form. The aims and nature of the study and the procedures they would need to undergo if they were enrolled in the study was explained to the potential participant. Informed consent from all eligible arsenicosis patients was obtained.

From the same household or from the neighborhood individuals eligible for inclusion as study participant in 'Group 2' was sought, matching for age (±2 years) and gender. The aims and nature of the study, the procedures that they would need to undergo if they were enrolled in the study was explained to the potential participant and their consent to participate in the study was obtained.

For selection of participants for the non-exposed group (reference group) an upazilla known to have least contamination in terms of proportion of tubewells contaminated was selected. Households having eligible participants in terms of age and gender were approached, and their eligibility to be study participants was assessed using the pretested eligibility form. No more than two individuals from a household were included in the study. The aims and nature of the study, and the procedures they would need to undergo if they were enrolled in the study were explained to the potential participants, and finally consent from eligible household members was obtained.

3.9 DATA COLLECTION

3.9.1 PREPARATION FOR DATA COLLECTION:

The designed research instruments were initially field tested in Samta village of Jessore in 2005 (March)[388]. Based on initial field test observations they were modified and were again field tested at Sonargaon and thereafter they were finalized.

Data collection was initially implemented for groups I & II. Thereafter data collection for Gr III was carried out. For each upazilla a list of arsenicosis patients in the age range of 30 to 60 years was prepared based on the collected patient lists and the patients were sorted by bari, para, village, ward/mouja and union.

Before the data collection was initiated a team of physicians and paramedics (medical technologists) was formed and oriented with the processes and tools of data collection to be utilized in medical camps the study.

3.9.2 HOUSEHOLD DATA COLLECTION:

In each of the upazilla, a separate panel of data collectors was recruited through the local health authority for household data collection. Just prior to the commencement of data collection in each upazilla, a two day orientation for data collectors was arranged to orient them with the objectives of the study, the study process and the forms and questionnaires that would be used for data collection. Hands on training on application of different research instruments [Information to participant, Participant Eligibility Screening Form(s), Consent form, Participant log (annex-8), Household Questionnaire, Age matching chart, Referral slip (annex-9)], use of ID numbers, technique of water sample collection and labeling and the means of recruiting participants of Gr I and Gr II in Alamdanga, Shahrasti and Lakhsam upazillas and participants for Gr III in Pakundia upazilla.

Each data collector was provided with adequate logistics for participant screening and recruitment, instrument for collection of household information (household questionnaire), containers for water sample collection, bags and waterproof marking pens, etc. They were also provided with specific dates for referral of consenting participants whose household interview had been completed to medical camps and a maximum number for referral on each date was also given.

Recruitment of Participants for Group I and data collection:

At the end of the training each data collector in Alamdanga, Shahrasti and Lakhsam upazillas were assigned villages in which there were to undertake data collection and was provided with a list of arsenicosis patients residing in the villages to which they had been assigned. Each data collector visited the households of listed arsenicosis patients, informed the individuals of the purpose of his/her visit using the "information for participants" which included the aims and nature of the study and the procedures a participant would need to undergo if he/she were enrolled in the study. Any arsenicosis patient who expressed his/her interest to become a study participant was then assessed for eligibility to be a study participant of Gr I. If eligible, the participant was asked to

provide consent for participation in a prepared format (consent form) and was assigned an ID number from the list of ID numbers provided to the data collector. Subsequently the consenting individual was interviewed using household interview schedule to elicit sociodemographic information; selected lifestyle information; food consumption frequency; residency history and information on source(s) of water for drinking and cooking purpose. During this visit tubewell(s) and other sources used by the participant as source of drinking water was traced and water sample(s) from the source(s) were collected in supplied water sample container(s), and the container(s) were labeled using the ID number assigned for the selected participant. For sources that had been used by a study participant but had become non-existent, water samples of three (3) existing tubewells closest to the site where the non-existent water source was located was collected as proxy for the non-existent source. Then the ID number, age and gender of the participant were entered in the study participant log. The selected participant who had completed the household interview was then provided with a referral slip and was asked to attend the medical camp at 8 AM on an assigned date. The referred participant was especially asked to refrain from eating or drinking anything except water since waking up on the day he/she was asked to attend the medical camp.

Recruitment of Participants for Group II and data collection:

From the same household or from the neighborhood individuals eligible for inclusion as study participant in 'Group 2' would be sought matching for age (±2 years) and gender. Subsequently informed consent was obtained and data collection was completed in accordance to the process described above.

Recruitment of Participants for Group III and data collection:

At the end of the training in Pakundia upazilla, each data collector were assigned villages in which there were to undertake data collection and was provided with a list that included the age and the gender of participants they would be required to recruit from their assigned villages.

Each data collector visited the households in their assigned village(s), checked out if any of the resident's age and gender matched with the gender and age (±2 years) included in the list provided. If a match was found the data collector informed the individual(s) of the purpose of his/her visit using the "information for participants" which included the aims

and nature of the study and the procedures a participant would need to undergo if he/she were enrolled in the study. Any household member who expressed his/her interest to become a study participant was then assessed for eligibility to be a study participant of Gr III. If found eligible, the participant was asked to provide consent for participation in a prepared format (consent form) and was assigned an ID number from the list of ID numbers provided to the data collector. Thereafter data collection proceeded in accordance to that as described above (recruitment of participants for group I and data collection).

3.9.3 DATA COLLECTION AT MEDICAL CAMP:

All participants attending the medical camp were asked to undergo the planned medical history taking and medical examination. Participants attending the medical camp in fasting state were initially asked to provide finger prick capillary blood for estimation of blood glucose level with the aid of Accu Chek Active[389]. Then they were subjected to glucose load (75 gm anhydrous glucose dissolved in 250ml water). And a capillary blood glucose level 2 hour after glucose load was obtained. Measurement of standing height and weight (with minimal clothing and without shoes) for estimation of BMI was then undertaken. This was followed by assessment of blood pressure status by measuring the blood pressure in the right arm in sitting position following 5 minutes of rest, following the WHO protocol[243]. Three measurements were taken with approximately 3 minutes intervals between each measurement. Averaged systolic and diastolic pressures of two closely matching measurements were taken as systolic and diastolic blood pressures of the participant and were utilized in assessing the blood pressure status of the participant.

The ABI assessment[14,21,23-25,29,30,228,229] was undertaken utilizing the Nicolet Vascular ABI kit having a 5 MHz broadband probe and a random zero sphygmomanometer. For the purpose the participant was asked to rest in supine position without shoes and stockings/socks for 5 minutes with blood pressure cuff of the ABI kit snugly applied on both upper arms and legs. The cuffs were applied in such a manner that the lower edge of the cuffs applied to the upper arms were about midway up the upper arms above the elbows and the lower edge of the cuffs applied to the lower ends of the legs were just above the ankles. The measurements of were carried out as described below.

1. The sphygmomanometer was connected to the cuff applied over the right arm.

2. The right brachial artery was located and a 1-2 cm ribbon of Doppler ultrasound gel was applied over the location (it was ensured that sufficient gel had been applied).
3. Turning on the Doppler probe was placed at the right antecubital area in soft contact with the skin. Then the probe was moved around until the clearest arterial pulse sounds was heard.
4. Keeping the probe in that position the blood pressure cuff was inflated to approximately 30 mm Hg above the reading where the pulse sound had ceased.
5. Then the cuff was then deflated slowly (at a rate of 2 mm Hg per second) until the first arterial sound was heard. The pressure reading that coincided with the appearance of the first arterial sound was recorded and the cuff was then completely deflated. Thus the right brachial systolic pressure was obtained.
6. Then the sphygmomanometer was disconnected from the cuff.
7. The gel applied to the participant's skin was then removed with a tissue.
8. Subsequently the sphygmomanometer was connected to the cuff applied to the right ankle and the participant was asked to rotate the right foot outwards.
9. A 1-2 cm ribbon of Doppler gel was then applied to the area immediately adjacent to the medial malleolus (ensuring that sufficient gel had been applied).
10. Turning on the Doppler probe, it was then placed near the ankle's center close to the margin of the medial malleolus and moved around until the clearest arterial sound was heard. Keeping the probe in position the steps 4, 5 and 7 were repeated. Thus the right tibialis posterior systolic pressure was obtained.
11. Then the participant was asked to rotate the right foot to neutral position and a 1-2 cm ribbon of Doppler gel was applied to the area between the big toe and 2nd toe halfway down the foot (ensuring that sufficient gel had been applied).
12. Turning on the Doppler probe, it was then placed over the area between the big toe and 2nd toe halfway down the foot and moved around until the clearest arterial sound was heard. Keeping the probe in position steps 4-7 was repeated. Thus the right dorsalis pedis systolic pressure was obtained.

In the way described above, systolic pressures at the left tibialis posterior, left dorsalis pedis and finally at the left brachial artery were obtained. These systolic pressure measurements were utilized for estimation of ABI as a marker of LEAD.

On completion of all activities and procedures planned for the medical camp the relevant questionnaires and checklist(s) were checked for completeness before the participant departed.

Subsequently the households of the participants who had completed the medical examination were revisited and during this visit tubewell(s) and other sources used by the participant as source of drinking water were traced and water samples for estimation of arsenic content were collected in pretreated 100 ml polyethylene bottles. During this visit any detected discrepancy in previously undertaken data collection was rectified.

3.9.4 ESTIMATION OF ARSENIC IN WATER SAMPLES

Water samples, collected from water sources used by the participants were transported to NIPSOM-DOEH arsenic lab for estimation of total arsenic. Analysis of total arsenic in the NIPSOM-DOEH arsenic lab was carried out with Millennium Excalibur PSA 10.055 coupled with an autosampler and driven by Avalon windows software and a control computer. The methodology of arsenic estimation was in line with the PSAnalytical Application Note 017[390] in which gaseous hydride (arsine) is generated by treating the acidified sample solution with sodium tetrahydroborate covalent and the hydride was atomized and the resulting atoms detected by atomic fluorescence spectrometry.

3.10 DATA MANAGEMENT AND ANALYSIS

At the end of each day's work all questionnaires and checklists were reviewed for accuracy, consistency and completeness. Attempt was undertaken to rectify the incomplete questionnaires through follow-up household visits as required. As for checklists relating to medical examinations each was checked for completeness before the participant left the place of medical examination.

Data obtained would be entered in SPSSWIN 11.5 program[391] for analysis. ABI was estimated through the utilization of systolic pressure obtained through the use of Doppler the Nicolet Vascular ABI Kit[21,28,228]. For each study participant the higher of the two brachial pressures was taken as the brachial systolic pressure and for each lower

extremity the higher of the two ankle systolic pressures (posterior tibial and dorsalis pedis arteries) was taken as the ankle systolic pressure. Then the ABI for each lower extremity was calculated by dividing the ankle systolic pressure by the ankle systolic pressure. Any study participant having an ABI of 0.90 or less for any of the lower extremity was considered as having LEAD. For each participant the BMI, HTN status, Diabetic status and the cumulative arsenic exposure (mg-years/L) were determined. Analysis of data was carried out in line with the objectives of the study. Univariate, bivariate and multivariate analyses were carried out using both descriptive and inferential statistics. The comparability of the groups in terms of sociodemographic characteristics were assessed using Student's t test, χ^2 test and other appropriate tests as applicable. The data were stratified by potential confounders (age, gender, smoking status, obesity, hypertension and diabetes) and subsequently analyzed making adjustments for the confounders. Adjusted prevalence rates were calculated through direct standardization method and for doing so a standard population was constructed by pooling the strata specific distribution of participants of all the three groups. Odds ratios (ORs) and adjusted ORs and their 95% confidence interval (CI) were obtained through the binary logistic regression procedure.

3.11 ETHICAL ISSUES

Participation in the study was completely on voluntary basis. Prior to enrolment as participants each potential participant was provided with written information in Bangla (annex-IV) regarding study. For those who were illiterate, the information was read out. The content of the information sheet was designed so as to explain about the aims and nature of the study and the procedures a participant would need to undergo if he/she was enrolled in the study. The participants were also informed that even if they consented to be a participant of the study they would have the right to refuse to respond to any or all of the interview questions and may decline to provide a blood, urine, as well as environmental samples. Each potential participant was be enrolled as a study participant after they had provided consent by putting their signature or thumb impression on the consent form (annex-V).

The enrolled participants was be provided with assurance that information acquired in course of the study will not be released to any other individual unless authorized by the

participant and that the information gained would be used in such a way that identity of the participant would not be revealed.

No experimental drugs or placebos were administered as part of this study.

All tests planned for the study were of non-invasive nature except for the procedure for collecting blood sample for glucose estimation. As finger prick capillary blood was used in blood glucose estimation it was minimally invasive. In obtaining finger prick capillary blood the finger was dapped with antiseptic and pricked with a single-use lancet. Therefore the risks arising out of the procedure could include some discomfort, a punctured wound and rarely infection. So it is considered highly unlikely that the participants would require emergency care in relation to providing the biological sample (finger prick blood) required for the study. In case if medical care was needed arrangements for transportation of the participant to a local health center or hospital for appropriate care was made. This risk coverage by the researcher was clearly spelled out to the participants during the recruitment process.

The primary purpose of the study was to provide information if individuals exposed to arsenic through drinking water (arsenic at levels <0.05 mgL^{-1}) were at higher risk of LEAD compared to non-exposed individuals (water containing arsenic at levels <0.05 mgL^{-1}). In doing so, in addition to assessment for LEAD other health effects, like obesity, hypertension, diabetes mellitus, etc., was assessed. The results of all such assessments were shared with the participants on individual basis and if necessary, were advised to seek appropriate medical help.

3.12 LIMITATIONS IN THE STUDY:

1. Due to superstitions surrounding chronic disabling diseases especially as in the cases of arsenicosis, families are often not only reluctant to give information about afflicted household members but also often refrain from attending places where they would be examined for skin lesions of arsenicosis where participants without such lesions would also be attending. This attitude could have influenced the recruitment of and participation by subjects with severe skin lesions; if more individuals with severe skin lesions would have been recruited the findings of the study would have been attenuated rather than strengthening the observed effects.

2. Selection bias could have occurred if participants whether of arsenic-exposed or of reference groups suffering from diabetes mellitus or hypertension were somehow more easily recruited to the study.
3. It is possible that there was some information bias in the household and personal information collected by interviews through household visits.
4. For practical reasons, it was necessary to carry out screening for diabetes mellitus by testing capillary blood for glucose levels rather than using the conventional standard method of using venous blood determining diabetic status.
5. Dyslipidemia is considered as a risk factor for LEAD, but in this study it was not taken into consideration as it was observed that arsenic exposure did not affect the lipid levels in human populations exposed to excess arsenic and lipid levels besides being in the normal none of the parameters in those having PVD was different from those not having PVD. Rather it was assumed that individuals with blood lipid abnormalities had an equal possibility of being recruited in all of the three study groups.
6. Problems of quantification and retrospective assessments of exposures were problematic as usual. Verifiable records of information on tubewells not to mention of the arsenic content of water yielded by those tubewells used by the targeted rural population in the study areas in their life time were not available.
7. Moreover it would have been desirable to have directly measured individual exposure data over time, because the available water samples reflected only a particular point in time and not the historical exposure and possible fluctuations in arsenic concentrations in time. Arsenic concentrations have been reported to vary over time from well to well in the same area, and even from season to season within the same well. In this current study it was not possible to take into account of such variation.
8. Humic substances have been mentioned as a possible cause of blackfoot disease and diabetes mellitus. However, to date no epidemiologic evidence had been presented to show a correlation between exposure to humic substances and development of PVD and the association between humic substances and BFD if any, might be due to the confounding effect of the strong association between

BFD and arsenic exposure. Humic acids are a group of polymers with high molecular weights that results from the decomposition of organic matter, particularly dead plants. Though not reported, it is possible that humic acid could be present in the groundwater tapped by hand tubewells in Bangladesh. Humic acid if present in ground water of Bangladesh would be present in tubewell water of participants of all of the three groups. Thus if humic acid had any role in inducing LEAD, all of the groups are most likely to have been similarly affected and hence are least likely to have influence the findings of the study.

CHAPTER IV

RESULTS

4.1 COMPARISON OF STUDY GROUPS BY STUDY VARIABLES

Table- 17: Distribution of participants by gender and study groups

Gender	Study Groups			Total	Significance
	Gr I	Gr II	Gr III		
Male	433 (43.3)	403 (40.3)	404 (40.4)	1240 (41.3)	$\chi^2 = 2.395$; df = 2, p= 0.302
Female	567 (56.7)	597 (59.7)	596 (59.6)	1760 (58.7)	
Total	1000	1000	1000	3000	

Males constituted 41.3% (1240) of the study participants. Individually males constituted 43.3% (433), 40.3% (403) and 40.4% (404) of the study participants of Gr-I (arsenicosis group), Gr-II (arsenic exposure only group) and Gr-III (reference group) respectively. The study groups were not statistically different in terms of gender ($\chi^2 = 2.395$; df = 2, p= 0.302).

Table-18: Distribution of respondents by age and study group

Age group (years)	Study group			Total	Significance
	Gr I	Gr II	Gr III		
30-39	494 (32.4) (49.4)	549 (36.0) (54.9)	482 (31.6) (48.2)	1525 (50.8)	χ^2 =12.808 df=4; p=0.012
40-49	356 (37.5) (35.6)	298 (29.9) (29.8)	344 (34.5) (34.4)	998 (33.3)	
50-60	150 (31.4) (15.0)	153 (32.1) (15.3)	174 (36.5) (17.4)	477 (15.9)	
Total	1000	1000	1000	3000	

The minimum and maximum age of the respondents was 30 and 60 years respectively. Out of the 3000 study participants almost 50.8% (1525) were between 30 to 39 years of age and only 15.9% (477) were between 50 to 60 years of age. Most of the respondents in each of the study groups (Gr-I 49.4%, Gr-II 54.9% and Gr-III 48.2%) were between 30 to 39 years of age. Fewer numbers of participants in all the groups (15% in Gr-I, 15.3% in Gr-II and 17.4% in Gr-III) were between 50-60 years of age.

Table- 19: Comparison of age of participants by gender and study groups

Gender	Study Groups	N	Age of participants (years)		Significance
			Mean ± SD	95% CI	
Male	Group I	433	41.03±8.021	40.28-41.79	F=2.999, df 2; p=0.05
	Group II	403	41.06±8.021	40.25-41.87	
	Group III	404	42.26±8.151	41.46-43.05	
	All Groups	1240	41.44±8.165	40.99-41.90	
Female	Group I	567	39.59±7.120	39.01-40.18	F=0.186, df 2; p=0.831
	Group II	597	39.58±7.083	39.02-40.15	
	Group III	596	39.81±7.647	39.20-40.43	
	All Groups	1760	39.66±7.287	39.32-40.00	
Both sexes	Group I	1000	40.22 ±7.554	39.75- 40.69	F=2.036, df 2; p=0.131
	Group II	1000	40.18 ±7.624	39.71-40.65	
	Group III	1000	40.80 ±7.941	40.31-41.29	
	All Groups	3000	40.40 ±7.711	40.12-40.68	

The mean age (and 95% CI) of the respondents of Gr-I, Gr-II and Gr-III were 40.2 ± 7.6 (39.8-40.7), 40.2 ± 7.6 (39.7-40.7) and 40.8 ±7.9 (40.3-41.3) years respectively. The study groups were statistically similar in terms of age of the participants (F= 2.036; p = 0.131).

The mean age of the male participants were 41.03±8.021, 41.06±8.021 and 42.26±8.151 years for participants in Gr-I, Gr-II and Gr-III respectively. The mean age of respondents of male participants was slightly higher in Gr-III than in the other two groups and the difference was statistically just significant (F=2.999; p= 0.05).

On the other hand the mean age of the female participants were 39.59±7.120, 39.58±7.083 and 39.81±7.647 years for participants in Gr-I, Gr-II and Gr III respectively. Though the mean age of respondents of female participants of the different groups varied slightly, there was no statistically significant difference (F=0.186; p= 0.831).

Table- 20: Comparison of duration of participant's residence in the current household by gender and study groups

Gender	Study Groups	N	Duration of residence in the current household (years)		Significance
			Mean ± SD	95% CI	
Male	Group I	433	41.03±8.019	40.27-41.79	F =2.955, df 2; p=0.0524
	Group II	403	41.04±8.278	40.23-41.85	
	Group III	404	42.23±8.158	41.43-43.03	
	All Groups	1240	41.42±8.162	40.97-41.88	
Female	Group I	567	23.61±7.224	23.01-24.20	F =2.218, df 2; p=0.109
	Group II	597	23.22±7.268	22.64-23.80	
	Group III	596	22.69±7.867	22.06-23.32	
	All Groups	1760	23.17±7.468	22.82-23.52	
Both sexes	Group I	1000	31.15±11.49	30.44-31.86	F =1.084, df 2; p=0.338
	Group II	1000	30.40± 11.64	29.68-31.12	
	Group III	1000	30.59±12.48	29.81-31.36	
	All Groups	3000	30.71±11.89	30.29-31.14	

The mean duration of stay of the study participants of Gr-I, Gr-II and Gr-III in the current household were 31.15 (±11.49), 30.59(±12.48) and 30.59(±12.48) years respectively; and no statistically significant difference was detected (F=1.084; p>0.05).

When males and females were considered separately it was found that the mean duration of stay of the male study participants of Gr-I, Gr-II and Gr-III in the current household were 41.03 (±8.019), 41.04 (±8.278) and 42.23 (±8.158) years respectively and it was not found to be statistically significant (F=2.955; p>0.05).

As for the female study participants the mean duration of stay in the current household were 23.61 (±7.224), 23.22 (±7.268) and 22.69 (±7.867) years for those included in of Gr-I, Gr-II and Gr-III respectively. And any differences between the groups were not found to be statistically significant.

Table-21: Distribution of study participants by religion and study groups

Religion	Study groups			Total	Significance
	Gr I	Gr II	Gr III		
Islam	984 (98.4)	990 (99.0)	983 (98.3)	2957 (98.6)	$\chi^2 = 2.029$, df = 2; p= 0.363
Hinduism	16 (1.6)	10 (1.0)	17 (1.7)	43 (1.4)	
Total	1000	1000	1000	3000	

Out of the 3000 study participants 98.6% (2957) were followers of Islam while the rest (1.4%) were Hindus. The proportion of Hindus in the different study groups were 1.6%, 1.0% and 1.7% in Gr-I, Gr-II and Gr-III respectively. The groups were statistically similar ($\chi^2 = 2.029$, df = 2; p= 0.363) in terms of the religion of the participants.

Table-22: Distribution of study participants by marital status and study groups

Marital status	Study groups			Total	Significance
	Gr I	Gr II	Gr III		
Married	960 (96.0)	964 (96.4%)	977 (97.7%)	2901 (96.7%)	$\chi^2 = 4.951$; df = 2 p= 0.084
Divorced/Widow/Widower	40 (4.0)	36 (3.6)	23 (2.3)	99 (3.3)	
Total	1000	1000	1000	3000	

Out of the 3000 study participants 96.7% (2901) were married at the time of study. The rest (3.3%) were at that point of time single as they had underwent the process of divorce or were widow or widower.

Moreover the groups were statistically similar ($\chi^2 = 4.951$; df = 2 p= 0.084) in terms of marital status of the study participants.

Table-23: Comparison of study groups by household size of participants

Study Groups	N	Household size		Significance
		Mean ± SD	95% CI	
Group I	1000	6.45±1.179	6.38-6.53	F =2.596, df 2; p =0.075
Group II	1000	6.44±1.183	6.37-6.51	
Group III	1000	6.55±1.020	6.48-6.61	
All Groups	3000	6.48±1.131	6.44-6.52	

The minimum household size of the study participants was 4 whereas the maximum was 10. The mean household size of the participants was 6.48±1.131. The mean household size for Gr-I, Gr-II and Gr-III were 6.45±1.179, 6.44±1.183 and 6.55±1.020 respectively. Moreover no statistically significant difference (F=2.956; p=0.075) in household size between the study groups were found detected.

Table-24: Distribution of study participants by schooling and study groups.

Formal education (Ever went to school)	Study Groups			Total
	Gr I	Gr II	Gr III	
Yes	567 (56.7)	548 (54.8)	534 (53.4)	1649 (55.0)
No	433 (43.3)	452 (45.2)	466 (46.6)	1351 (45.0)
Total	1000	1000	1000	3000

Of the total 3000 study participants 55% had reported that they had received schooling (formal education). The number of participants reporting of having received some level of schooling was consistently high in all the groups. And no statistically significant difference between the groups (χ^2 =2.217, df =2; p= 0.330) in terms of schooling was detected.

Table-25: Distribution of study participants by gender, occupation and study groups.

Gender	Occupation	Study groups			Total	Significance
		Gr I	Gr II	Gr III		
Male	Agriculture/Farming	235 (54.3)	237 (58.8)	227 (56.2)	69 (56.4)	χ^2=12.218, df 10; p = 0.271
	Rickshaw Van puller/ Boatman/ Daily laborer/Fisherman	65 (15.0)	64 (15.9)	44 (10.9)	173 (14.0)	
	Business/ Shop keeper/ Hawker	92 (21.2)	64 (15.9)	88 (21.8)	244 (19.7)	
	Salaried job	30 (6.9)	27 (6.7)	31 (7.7)	88 (7.1)	
	Retired/ Pensioner	9 (2.1)	8 (2.0)	8 (2.0)	25 (2.0)	
	Painter/ Cloth-textile dyer	2 (0.5)	3 (0.7)	6 (1.5)	11 (0.9)	
	Total	433	403	404	1240	
Female	House wife/ Household work	555 (97.9)	590 (98.8)	585 (98.2)	1730 (98.3)	χ^2=1.653, df 2; p=0.438
	Salaried job	12 (2.1)	7 (1.2)	11 (1.8)	30 (1.7)	
	Total	567	597	596	1760	

Most of the male participants (≥54.3%) in the all the study groups were involved in agriculture or farming. Business-shop keeping-hawker was the second largest occupational group among m ale participants of all the study groups ((15.9~21.8%). Small proportions (2.0-2.1%) of the participants in the different groups were either pensioners or had given up their occupation (retired). The study groups were found not to be statistically different (χ^2 =12.218, df 10; p>0.05) in terms of composition by occupation. Similarly among female study participants the groups in terms of composition by occupation was found not to be statistically different (χ^2 1.653, df 2; p>0.05).

Table- 26: Distribution of participants by household possession of agricultural land and study groups

Household possession of agricultural land (Bigha)	Study groups			Total	Significance
	Gr I	Gr II	Gr III		
None	333 (33.3)	400 (40.0)	307 (30.7)	1040 (34.7)	χ^2=29.2976 df 4; p<0.0001
<3	380 (38.0)	392 (39.2)	422 (42.2)	1194 (39.8)	
3-25	287 (28.7)	208 (20.8)	271 (27.10	766 (25.5)	
Total	1000	1000	1000	3000	

Almost 35% of the study participants were from household with no agricultural land and only 25.5% were from households having 3 to 25 bighas of land. Higher proportion (40%) of participants in Group II had no cultivatable land compared to that for other groups (33.3% in Gr-I and 30.7% for Gr-III). Fewer (20.8%) participants of Gr-II were from households having 3-25 bighas of agricultural land compared to participants of the other groups (28.7% for Gr-I and 27.1% for Gr-III). Moreover the groups were found to be statistically different (χ^2=29.2976, df 4; p<0.05) in terms of household possession of agricultural land.

Table-27: Distribution of participants by type of housing and study group

Type of housing	Study groups			Total	Significance
	Gr I	Gr II	Gr III		
Kacha floor+ Thatched walls+ Thatched roof	8 (0.8)	10 (1.0)	7 (0.7)	25 (0.8)	χ^2=13.768, df=10 ; p=0.184
Kacha floor+ Thatched walls+ Tin roof	103 (10.3)	104 (10.4)	97 (9.7)	304 (10.10	
Kacha floor+ mud walls+ Tin/Tally roof	639 (63.9)	623 (62.3)	683 (68.3)	1945 (64.8)	
Pucca floor+ Tin walls+ Tin/Tally roof	179 (17.9)	207 (20.7)	159 (15.9)	545 (18.2)	
Pucca floor+ Pucca walls+ Tin/Tally roof	62 (6.2)	46 (4.6)	46 (4.6)	154 (5.1)	
Pucca floor+ Pucca walls+ Pucca roof	9 (0.9)	10 (1.0)	8 (0.8)	27 (0.9)	
Total	1000	1000	1000	3000	

Highest proportion of the study participants in all the groups (Gr I- 63.9%, Gr II- 62.3% and Gr III-68.3%) lived in households having kacha floor, mud walls and tin or tally roof. Only 0.8% of the study participants lived in houses having kacha floor, thatched walls and thatched roof (Gr I- 0.8%, Gr II- 1% and Gr III- 0.7%). More over less than 1% lived in pucca household (Gr I- 0.98%, Gr II- 1% and Gr III- 0.8%). And the groups were found not to statistically different (χ^2 =13.768, df 10; $p > 0.05$) in terms of type of their dwelling house.

Table- 28: Distributions of participants by household possession and study groups.

Household possession		Study groups			Total	Significance
		Gr I (N=1000)	Gr II (N=1000)	Gr III (N=1000)		
Tv in household	No	593 (59.3)	680 (68.0)	685 (68.5)	1958 (65.3)	χ^2=23.612 p<0.0001
	Yes	407 (40.7)	320 (32.0)	315 (31.5)	1042 (34.7)	
Radio or 2 in1 in household	No	709 (70.9)	748 (74.8)	602 (60.2)	2059 (68.6)	χ^2=53.087 p<0.0001
	Yes	291 (29.1)	252 (25.2)	398 (39.8)	941 (31.4)	
Bicycle in household	No	607 (60.7)	629 (62.9)	440 (44.0)	1676 (55.9)	χ^2=86.652 p<0.0001
	Yes	393 (39.3)	371 (37.1)	560 (56.0)	1324 (44.1)	
Motorcycle in household	No	913 (91.3)	948 (94.8)	910 (91.0)	2771 (92.4)	χ^2=12.661 p=0.002
	Yes	87 (8.7)	52 (5.2)	90 (9.0)	229 (7.6)	

Approximately 35% of the study participants (40.7% in Gr I, 32.0% in Gr II and31.5% in Gr III) had a television in the household. Radio or 2 in1 was possessed by 29.1%, 25.2% and 39.8% households of the participants of Gr-I, Gr-II and Gr-III respectively. Bicycle was possessed by 39.3%, 37.1% and 56.0% households of participants of Gr-I, Gr-II and Gr-III respectively. Among Gr-I, Gr-II and Gr-III participants 8.7%, 5.2% and 9.0% respectively had a motorcycle in the household.

Moreover the groups were found to be significantly different ($p<0.05$) in terms of household possession of television, radio or 2 in1, bicycle and motorcycle.

Table- 29: Annual household income (Taka) of study participants by study groups.

Study Groups	N	Annual household income		Significance
		Mean ± SD	95% CI of mean	
Group I	1000	92914.50 ± 42414.131	90282.50 - 95546.50	F= 0.265, df 2; p =0.767
Group II	1000	91415.21 ± 44100.45	88678.57 - 94151.85	
Group III	1000	92145.60 ± 51244.06	88965.67 - 95325.53	
All Groups	3000	92158.44 ± 46067.48	90509.30 - 93807.58	

The mean annual household income of participants of Gr-I, Gr-II and Gr-III were Tk 92914.50 (± 42414.131), Tk 91415.21 (± 44100.45) and Tk 92145.60 (± 51244.06) respectively. The minimum household income was Tk 30000 for participants of Gr-I and Gr-III, and Tk 32000 for participants of Gr-II. Whereas, the maximum household income for participants of Gr-I, Gr-II and Gr-III was Tk 280000, Tk 282000 and Tk 400000 respectively. More over the groups were found not to be statistically different ($p>0.05$) in terms of mean household income.

Table- 30: Distribution of participants by staple food and study groups.

Staple food	Study group			Total	Significance
	Gr I	Gr II	Gr III		
Rice	435 (43.5)	406 (40.6)	42 (4.2)	883 (29.4)	χ^2=1052.230, df 6; p <0.0001
Rice + Potato	31 (3.1)	56 (5.6)	84 (8.4)	171 (5.7)	
Rice + Pulses	369 (36.9)	319 (31.9)	115 (11.5)	803 (26.8)	
Rice + Pulses + Potato	165 (16.5)	219 (21.9)	759 (75.9)	1143 (38.10	
Total	1000	1000	1000	3000	

Rice was the most common staple food consumed by of participants of Gr-I (43.5%) and Gr-II (40.6%), while rice with pulses and potato was the most common staple food (38.1%) among participants of Gr-III. Moreover the groups were found to be statistically different in terms of staple food consumed by the participants (χ^2 = 1052.23, df 6; p < 0.05).

Table- 31: Distribution of participants by staple food type and study groups.

Staple food type	Study group			Total	Significance
	Gr I	Gr II	Gr III		
Starch based	466 (46.6)	462 (46.2)	126 (12.6)	1054 (35.1)	χ^2= 334.231 df 2; p<0.0001
Starch & Vegetable protein	534 (53.4)	538 (53.8)	874 (87.4)	1946 (64.9)	
Total	1000	1000	1000	3000	

The staple food consumed by study participants when grouped as starched based and a combination of starch and vegetable protein (dal), it was found that among the 3000 study participants 35.1% consumed starch based staple food. Though higher proportion of participants in each of the study groups consumed starch and vegetable protein based staple food the difference of type of staple food consumed by participants of Gr I and Gr II was marginal. On the other hand the difference among the participants of Gr III was substantially high. Moreover the type of staple food consumed by the participants of the different groups was found to be statistically significant (p<0.05).

Table-32: Distribution of participants by consumption of vegetables and study groups.

Vegetables consumption per week	Study group			Total	Significance
	Gr I	Gr II	Gr III		
< 7 days	40 (4.0)	72 (7.2)	0 (0.0)	112 (3.7)	χ^2=72.418, df 2; p<0.0001
7 days	960 (96.0)	928 (92.8)	1000 (100.0)	2888 (96.3)	
Total	1000	1000	1000	3000	

About 96% of the study participants consumed vegetables 7 days a week. About 93% to 100% of the participants of Gr I (96%), Gr II (92.8%) and Gr III (100%) consumed vegetables throughout the week. And the groups were found to be statistically different (χ^2 = 72.418, df 2; p< 0.05).

Table – 33: Distribution of participants by consumption of milk or milk products and study groups.

Milk or milk product consumption	Study group			Total	Significance
	Gr I	Gr II	Gr III		
0 days per week	710 (71.0)	800 (80.0)	792 (79.2)	2302 (76.7)	χ^2= 40.734 df 4; p<0.0001
1 to 3 days per week	14 (1.4)	28 (2.8)	19 (1.9)	61 (2.0)	
4 or more days per week	276 (27.6)	172 (17.2)	189 (18.9)	637 (21.2)	
Total	1000	1000	1000	3000	

Most (76.7%) of the study participants did not consume milk or milk products even a single day a week. About 28%, 17% and 19% study participants of Gr I, Gr II and Gr III respectively consumed milk or milk product ≥ 4days a week. Fewer participants of Gr I (1.4%) consumed milk or milk product 1 to 3 days a week than participants of Gr II (2.8%) and Gr III (1.9%). More over the groups were found to be statistically different (χ^2=40.734 df 4; p<0.05).

Table- 34: Distribution of participants by gender, smoking status and study groups.

Gender	Smoking status	Study group			Total	Significance
		Gr I	Gr II	Gr III		
Male	Nonsmoker	230 (53.1)	200 (49.6)	196 (48.5)	626 (50.5)	χ^2 =4.0, df=4 ; p=0.087
	Past smoker	27 (6.2)	31 (7.7)	16 (4.0)	74 (6.0)	
	Current smoker	176 (40.6)	172 (42.7)	192 (47.5)	540 (43.5)	
	Total	433	403	404	1240	
Female	Nonsmoker	567 (100.0)	597 (100.0)	596 (100.0)	1760 (100.0)	
	Total	567	597	596	1760	

None of the female participants in any of the groups were either current smokers or had smoked in the past. Among the males 43.5% were current smokers, 50.5% had never smoked and the remaining 6.0% were past smokers. The smoking status of the male participants between the different study groups was not found to be significantly different (χ^2 =4.0 df 4; p>0.05).

Table- 35: Distribution of participants by smoking status (ever/never smoker) and study groups and gender.

Gender	Smoking status	Study group			Total	Significance
		Gr I	Gr II	Gr III		
Male	Ever smoker	203 (33.1) (46.9)	203 (33.1) (50.4)	208 (33.9) (51.5)	614 (49.5)	χ^2= 1.946, df 2; p=0.378
	Never smoker	230 (36.7) (53.1)	200 (31.9) (49.6)	196 (31.3) (48.5)	626 (50.5)	
Female	Never smoker	567 (32.2) (100)	597 (33.9) (100)	596 (33.9) (100)	1760 (100)	
Total		1000	1000	1000	3000	

As a very few participants were past smokers (2.5%) the smoking status of the participants was reclassified as ever and never smokers for subsequent analysis. Among the total of 1760 female participants in the study groups none had ever smoked. But among the total of 1240 male participants in the three study groups almost half were either current or past smokers. Among the males there were fewer ever smokers in groups Gr I than in Gr II and Gr III. But the differences in the smoking status among the male participants in the different groups was not found to statistically significant ($p>0.05$).

Table- 36: Distribution of participants by their habit of use of chewing tobacco and study groups.

Habit of chewing tobacco (Jorda/Shada pata)	Study group			Total	Significance
	Gr I	Gr II	Gr III		
No	754 (75.4)	742 (74.2)	644 (64.4)	2140 (71.3)	χ^2 =35.614, df=2; p=0.000
Yes	246 (24.6)	258 (25.8)	356 (35.6)	860 (28.7)	
Total	1000	1000	1000	3000	

About 29% of the study participants had the habit of chewing tobacco. Among the participants of Gr I, Gr II and Gr III 26.6%, 25.8% and 35.6% respectively had the habit of use of chewing tobacco. More over the groups were found to be significantly different (χ^2=35.614, df 2; $p<0.05$) in terms of having the habit chewing tobacco.

Table- 37: Distribution of participants by nutritional status and study groups.

Body mass index (kg/m^2)	Study group			Total	Significance
	Gr I	Gr II	Gr III		
Normal (<25)	932 (93.2)	916 (91.6)	905 (90.5)	2753 (91.8)	χ^2 =5.095, df=4; p= 0.278
Overweight (≥25 but <30)	60 (6.0)	72 (7.2)	83 (8.3)	215 (7.2)	
Obese (≥30)	8 (0.8)	12 (1.2)	12 (1.2)	32 (1.1)	
Total	1000	1000	1000	3000	
Mean	19.47	19.67	19.60	19.58	F=0.991, df=2 p=0 .371
SD	3.05	3.20	3.36	3.21	
Minimum	14.01	14.34	14.00	14.00	
Maximum	33.97	32.89	35.74	35.74	

Among all the participants most (91.8%) had normal BMI, 7.2% were overweight and a few (1.1%) were obese. Obesity was less common among participants of Gr I (0.8%) than among participants of the other two groups (1.2%). The groups were found not to be statistically different (χ^2=5.095, df 4; p>0.05) in terms of nutritional status (BMI).

Table- 38: Distribution of participants by their diabetic status and study groups.

D M status	Study group			Total	Significance
	Gr I	Gr II	Gr III		
Normal	932 (93.2)	941 (94.1)	969 (96.9)	2842 (94.7)	χ^2 =14.925, df=2; p=0.001
Diabetic	68 (6.8)	59 (5.9)	31 (3.1)	158 (5.3)	
Total	1000	1000	1000	3000	

Among the study participants 0.2% in Gr 1, 0.1% in Gr II and 0.3% in Gr III were taking medication for diabetes mellitus at the time of data collection. Based on fasting capillary blood glucose level of ≥6.1 $mmolL^{-1}$ and history of taking medication for diabetes mellitus 6.8%, 5.9% and 3.1% participants of Gr I, Gr II and Gr III respectively were found to be diabetic. And the groups were found to be statistically different (χ^2 =14.925, df 2; p<0.05) in terms of diabetic status of the participants.

Table- 39: Distribution of participants by their blood pressure status and study groups.

Blood pressure status	Study group			Total	Significance
	Gr I	Gr II	Gr III		
Normotensive	589 (58.9)	615 (61.5)	733 (73.3)	1937 (64.6)	χ^2 =52.453, df=4; p<0.001
PreHTN	345 (34.5)	322 (32.2)	231 (23.1)	898 (29.9)	
Hypertensive	66 (6.6)	63 (6.3)	36 (3.6)	165 (5.5)	
Total	1000	1000	1000	3000	

Among all the participants only 1% had been found to be taking antihypertensive medication because of they had already been diagnosed as hypertensive. Of the total 3000 study participants 5.5% were found to be hypertensive and another 29.9% were prehypertensive. Hypertension was more common amongst participants of Gr I (6.6%) than among participants of Gr II (6.3%) and Gr III (3.6%). Prehypertension was also more common among participants of Gr I (34.5%) than among participants of either Gr II (32.2%) or Gr III (23.1%). More over the groups were found to be significantly different (χ^2=52.453, df 4; p<0.05) in terms of blood pressure status.

Table-40: Distribution of participants by arsenic level in their current drinking water source and study groups.

Arsenic level (mg/L)	Study group			Total	Significance
	Gr I (N=1000)	Gr II (N=1000)	Gr III (N=1000)		
≤0.01	44 (4.4)	26 (2.6)	48 (4.8)	118 (3.9)	χ^2=2840.6, df= 4; p<0.000
> 0.01 but <0.50	5 (0.5)	3 (0.3)	952 (95.2)	960 (32.0)	
≥0.50	951 (95.1)	971 (97.1)	0 (0.0)	1922 (64.1)	
Mean ±SD	0.251 ±0.131	0.259 ±0.081	0.021 ±0.007	F=2315.994; p<0.0001	
Minimum	0	0	0.002		
Maximum	0.640	0.560	0.045		

The arsenic level in the current water source of the participants varied from 0~0.640 mg/L for those of Gr I, 0~0.560 mg/L for those of Gr II and 0.02~0.045 mg/L for those of Gr III. More than 95% of the participants of Gr I at the time of data collection were collecting water from sources whose arsenic level was 0.05 mg/L or higher. About 97% of the participants of Gr II were found to be collecting drinking water from sources that had arsenic at levels was 0.05 mg/L or higher. No participant of Gr III was found to have a current drinking water source which had level exceeding 0.049 mg/L. The mean arsenic levels (mg/L) of the current water source of the participants of Gr I, Gr II and Gr III were 0.251 (±0.131), 0.259 (±0.081) and 0.021 (±0.007) respectively. Moreover the groups were significantly different ($p<0.05$) in terms of arsenic content of the water of their current drinking water source.

Table-41: Distribution of participants by arsenic level in their past drinking water source and study groups.

Arsenic level (mg/L)	Study group			Total (N= 2954)	Significance
	Gr I (N=991)	Gr II (N=974)	Gr III (N=989)		
≤0.01	0 (0.0)	1 (0.1)	44 (4.4)	45 (1.5)	χ^2 =2949.64 df= 4 ; p=0.001
> 0.01 but <0.05	0 (0.0)	0 (0.0)	945 (95.6)	945 (32.0)	
≥0.05	991 (100.0)	973 (99.9)	0 (0.0)	1964 (66.5)	
Mean ±SD	0.289 ±0.131	0.250 ±0.135	0.021 ±0.007	F=1767.296; p<0.0001	
Minimum	0.100	0.004	0.004		
Maximum	0.641	0.564	0.047		

Among all the study participants, 98.5% (2954) had collected drinking water from another source before switching to the current water source. None of the participant of Gr I had a past water source whose arsenic level was lower than 0.05 mg/L. The arsenic level of the water source of the participants of Gr I was 0.100~0.641 mg/L and the mean level was 0.289 (±0.131) mg/L. About 94% of the participants of Gr II had a previous drinking water source. Except for a single participant, 99.9% of the participants of Gr II had a source that yielded arsenic in excess of 0.049 mg/L. The arsenic level of the water source of the participants of Gr II was 0.004~0.564 mg/L and the mean level was 0.250 (±0.135). About 99% of the participants of Gr III had a previous drinking water source. The arsenic level of the water source of the participants of Gr III was 0.004~0.047 mg/L and the mean level was 0.021 (±0.007). The groups were significantly different ($p<0.05$) in terms of arsenic content of the water of their current drinking water source.

Table- 42: Distribution of participants by selected clinical features and study groups

Clinical feature		Study group			Total	Significance
		Gr I	Gr II	Gr III		
Clinical anaemia	No	733 (73.3)	773 (77.3)	936 (93.6)	2442 (81.4)	χ^2 =154.67, df=4; p<0.0001
	Mild	250 (25.0)	218 (21.8)	61 (6.1)	529 (17.6)	
	Moderate	17 (1.7)	9 (0.9)	3 (0.3)	29 (1.0)	
Non purulent conjunctival congestion	No	996 (99.6)	999 (99.9)	1000 (100.0)	2995 (99.77)	
	Yes	4 (0.4)	1 (0.1)	0 (0.0)	5 (0.2)	
Pedeal edema	none	993 (99.3)	1000 (100.0)	1000 (100.0)	2993 (99.77)	
	Non pitting	6 (0.6)	0	0	6 (0.2)	
	Pitting	1 (0.1)	0	0	1 (0.03)	
Skin manifestation of arsenicosis	None	0	1000 (100.0)	1000 (100.0)	2000 (66.7)	
	Melanosis	465 (46.5)	0	0	465 (15.5)	
	Melanosis & Keratosis	535 (53.5)	0	0	535 (17.8)	
Amputation of limb or part	No	997 (99.7)	1000 (100.0)	1000 (100.0)	2997 (99.9)	
	Yes	3 (0.3)	0	0	3 (0.1)	

None of the participants in any of the study groups had central cyanosis or peripheral cyanosis; dehydration; palpable liver, spleen or intra-abdominal mass; and gangrene of lower limb. Clinical anemia was found to be more common among participants of Gr I (26.7%) than among those of Gr II (22.7%) and Gr III (6.4%). And the groups were found to be significantly dissimilar (χ^2 =154.67, df 4; p<0.05) in terms of clinical anemia amongst the participants.

Only 0.2% of the participants had non-purulent conjunctival congestion. And it was present amongst participants of Gr I (0.4%) and Gr II (0.1%).

A very few, 6 (0.23%) of the study participants had pedal edema. It was found only amongst the participants of Gr I, and non pitting edema (0.6%) was found to be more common occurrence than pitting edema (0.1%).

Melanosis of trunk (both front & back) or keratosis (both palm & sole) was found only amongst participants of Gr I, 46.5% had only melanosis while another 53.5% had both melanosis and keratosis.

Only participants of Gr I was found to have had amputation of part of limb (0.3%). And in all these case the amputations were surgical in nature and were located in the lower limb (2 cases having amputation of 1 or more fingers or digits of finger(s) of lower limb and 1 case with amputation at mid tarsal level).

Table-43: Distribution of participants by ABI and study groups

ABI	Study group			Total	Significance
	Gr I	Gr II	Gr III		
≤0.90 (LEAD)	166 (16.6)	89 (8.9)	35 (3.5)	290 (9.66)	Fisher's Exact Test value =104.586 p<0.0001
0.91-1.30 (Normal)	834 (83.4)	910 (91.0)	965 (96.5)	2709 (90.31)	
>1.30	0 (0.0)	1 (0.1)	0 (0.0)	1 (0.03)	
Total	1000	1000	1000	3000	
Mean ±SD	0.97 ± 0.09	0.98 ± 0.09	1.01 ± 0.08	0.99 ± 0.09	F=42.267, df 2; p<0.0001
Minimum	0.53	0.62	0.67	0.53	
Maximum	1.30	1.45	1.30	1.45	

Of the total 3000 study participants 9.66% had an ABI value which was ≤0.90 and only 0.03% had an ABI value which was higher than 1.30. Among participants in Gr I, Gr II and Gr III the proportion of participants with an ABI value of ≤0.90 was 16.6%, 8.9% and 3.5% respectively, and only 0.1% in Gr II had an ABI value that was greater than 1.30. The mean ABI among the all of the study participants was found to be 0.99 (± 0.09). The mean ABI for participants in Gr I, Gr II and Gr III was found to be 0.97

(±0.09), 0.98 (±0.09) and 1.01 (±0.09) respectively. The mean ABI for Gr I and Gr II was significantly lower ($p<0.05$) than the mean ABI for Gr III. Of the total 3000 study participants in three groups 1 (one) participant in Gr II had an ABI of >1.30, which implies that the particular participant had incompressible artery and hence was not included in all subsequent analyses.

4.2 LEAD BY STUDY GROUPS AND SOCIODEMOGRAPHIC VARIABLES

Table- 44: Distribution of LEAD among study participants by gender.

Gender	LEAD		Total	Significance	OR (95% CI))
	Yes	No			
Male	116 (9.4) (39.7)	1123 (90.6) (41.5)	1239 (41.3)	χ^2= 0.336, df 1; p=0.562	1
Female	176 (10.0) (60.3)	1584 (90.0) (58.5)	1760 (58.7)		1.076 (0.841-1.376)
Total	292 (9.7)	2707 (90.3)	2999		χ^2= 0.338, df 1; p=0.561

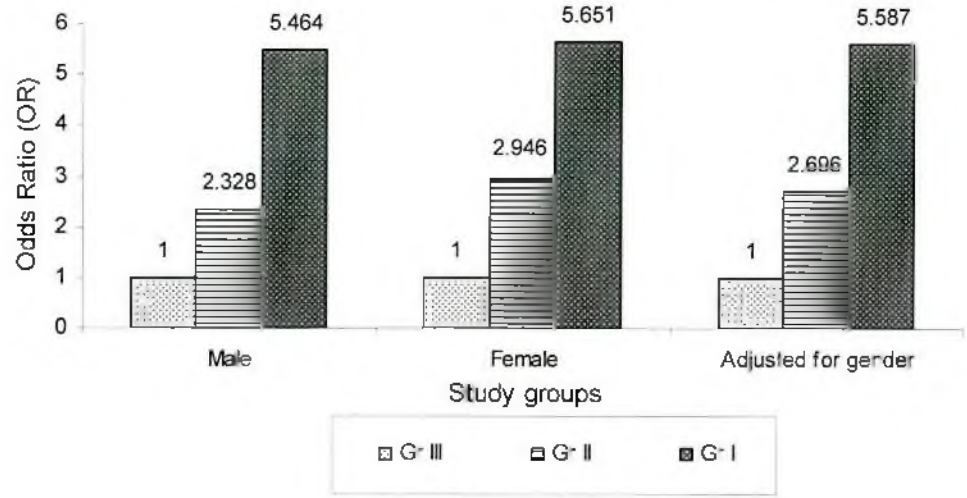
The male female ratio among the study participants was 100:142, amongst the 2999 study participants 41.3% (1239) were male and the remaining 58.7% (1760) were female. Of the 292 participants who had LEAD, 39.7% (116) were male and the rest 60.3% (176) were female. Though the prevalence of LEAD was found to be higher amongst female (10.0%) than amongst male (9.4%) the difference was not found to be statistically significant (χ^2= 0.336, df 1; p=0.562).

Moreover LEAD was found to be about 1.1 (0.8-1.4) times higher amongst female compared to that in males but it was not significant (χ^2= 0.338, df 1; p=0.561).

Table -45 : Distribution of LEAD among study groups by gender

Study group	Gender									OR (95% CI)		
	Male			Female			Combined					
	Total	LEAD		Total	LEAD		Total	LEAD		Male	Female	Adjusted for gender
		Yes	Prevalence (%)		Yes	Prevalence (%)		Yes	Prevalence (%)			
Gr I	433	71 (61.2)	16.4	567	97 (55.1)	17.1	1000	168 (57.5)	16.8	5.464 (3.026-9.864)	5.651 (3.472-9.199)	5.587 (3.836-8.136)
Gr II	402	31 (26.7)	7.7	597	58 (33.0)	9.7	999	89 (30.5)	8.9	2.328 (1.219-4.445)	2.946 (1.764-4.920)	2.696 (1.805-4.028)
Gr III	404	14 (12.1)	3.5	596	21 (11.9)	3.5	1000	35 (12.0)	3.5	1	1	1
Total	1239	116	9.4	1760	176	10.0	2999	292	9.7	χ^2= 15.07, df1; p<0.001	χ^2= 62.99, df 2; p<0.001	χ^2= 106.54, df 3; p<0.001
Significance	χ^2= 43.09, df 2; p<0.0001			χ^2= 59.65, df 2; p<0.0001			χ^2 =101.80, df=2; p<0.001					

Fig No-25 : LEAD among study groups by gender.



Lower Extremity Arterial Disease (LEAD) was found to be prevalent amongst 9.7% (292) of the study participants. Amongst those having LEAD 57.5% (168) were participants of Gr I, while the remaining 30.5% (89) and 12.0% (35) were participants of GR II and Gr III respectively. The prevalence of LEAD amongst Gr I, Gr II and Gr III participants were 16.8% (95% CI 14.5-19.1), 8.9% (95% CI 7.1-10.7) and 3.5% (95% CI 2.4-4.6) respectively. More over the groups were found to be significantly different (χ^2 =101.804, df 2; p<0.001) in terms of prevalence of peripheral arterial disease. Logistic regression revealed that LEAD was about 5.567 (3.823-8.107) times more likely amongst those whose drinking water arsenic content was >0.05 mg/L and who had arsenicosis (Gr I), and about 2.697 (1.805-4.028) times more likely to occur amongst those who have arsenic exposure (drinking water arsenic content >0.05 mg/L) but had not developed arsenicosis than those whose drinking water arsenic content was less than 0.05 mg/L (χ^2 =105.818, df 2; p<0.001).

Amongst the total 1239 male participants 34.9% (433), 32.5% (402) and 32.6% (404) belonged to Gr I, Gr II and Gr III respectively. The prevalence of LEAD amongst the male participants was found to be 9.4% (95%CI 7.7-11.0). Among the 116 male participants having LEAD most (61.2%) were members of Gr I, while the remaining 26.7% and 12.1% were members of Gr II and Gr III respectively. The prevalence of LEAD was highest (16.4%) among the participants of Gr I (95% CI 12.9-19.9) followed by that for Gr II (7.7%, 95% CI 5.1-10.3) and Gr III (3.5%, 95% CI 1.7-5.2). Moreover this difference in prevalence of LEAD among the male participants of the different study groups was found to be statistically significant (χ^2=43.099, df 2; p<0.05). Logistic regression revealed that LEAD was about 5.5 (3.0-9.9) times more likely amongst those males whose drinking water arsenic content was more than 0.05 mg/L and who had arsenicosis (Gr I), and about 2.3 (1.2-4.4) times more likely to occur amongst those who have arsenic exposure (drinking water arsenic content >0.05 mg/L) but had not developed arsenicosis than those whose drinking water arsenic content was less than 0.05 mg/L (χ^2= 15.075, df1; p<0.001).

Amongst the total 1760 female participants 32.22% (567), 33.92% (597) and 33.86% (596) belonged to Gr I, Gr II and Gr III respectively. The prevalence of LEAD amongst

the female participants was found to be 10.0% (95% CI, 8.6-11.4) . Among the 176 female participants having LEAD most (55.1%) were members of Gr I, while the remaining 33.0% and 11.9% were members of Gr II and Gr III respectively. The prevalence of LEAD was highest (17.1%) among the participants of Gr I (95% CI 14.0-20.2) followed by that for Gr II (9.7%, 95% CI 7.3-12.1) and Gr III (3.5%, 95% CI 2.0-5.0). The observed differences in the prevalence of LEAD among the female participants of the different study groups was found to be statistically significant (χ^2=59.657, df 2; p<0.05).

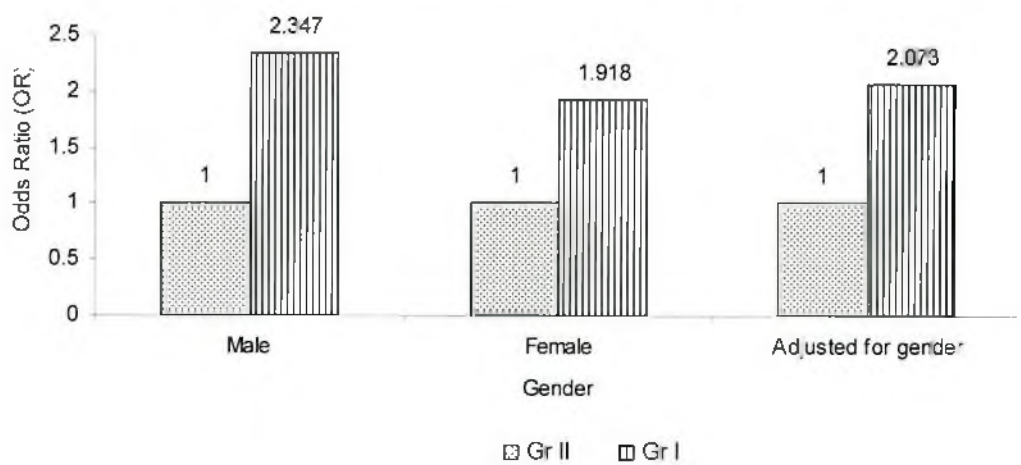
Logistic regression revealed that LEAD was about 5.6 (3.5-9.2) times more likely amongst those whose drinking water arsenic content was >0.05 mg/L and who had arsenicosis, and about 2.9 (1.8-4.9) times more likely (p<0.001) to occur amongst those who have arsenic exposure (drinking water arsenic content >0.05 mg/L) but had not developed arsenicosis, than those whose drinking water arsenic content was less than 0.05 mg/L (χ^2= 62.992, df 2; p<0.001).

Finally when adjusted for gender, LEAD was about 5.6 (3.8-8.1) times more likely amongst those whose drinking water arsenic content was >0.05 mg/L and who had arsenicosis (Gr I), and about 2.7 (1.8-4.0) times more likely to occur amongst those who have arsenic exposure (drinking water arsenic content >0.05 mg/L) but had not developed arsenicosis (Gr II), than those whose drinking water arsenic content was <0.05 mg/L (χ^2=106.546, df 3; p<0.001).

Table-46 : Distribution of LEAD among study groups I and II by gender

Study group	Gender									OR (95% CI)		
	Male			Female			Combined					
	Total	LEAD		Total	LEAD		Total	LEAD				
		Yes	Prevalence (%)		Yes	Prevalence (%)		Yes	Prevalence (%)	Male	Female	Adjusted for gender
Gr I	433 (51.9)	71 (69.6)	16.4	567 (48.7)	97 (62.6)	17.1	1000	168 (65.4)	16.8	2.347 (1.503- 3.667)	1.918 (1.354-2.716)	2.073 (1.576-2.726)
Gr II	402 (48.2)	31 (30.4)	7.7	597 (51.3)	58 (37.4)	9.7	999	89 (34.6)	8.9	1	1	1
Total	835	102	12.2	1164	155	13.3	1999	257	12.9	χ^2= 15.075, df1; p<0.001	χ^2= 13.868, df1; p<0.001	χ^2= 28.978, df 2; p<0.001
Significance	χ^2=14.666, df 1; p<0.001			χ^2=13.767, df 1; p<0.001			χ^2= 27.776, df 1; <0.001					

Fig No -26 : LEAD among study groups I and II by gender



Amongst the 1999 participants of Gr I and Gr II, 257 (12.9%) were found to have LEAD. Of them 168 were members of Gr I and the other 89 belonged to Gr II. Gr I participants having LEAD accounted for 8.4% of all participants and 65.4% of the participants having LEAD. On the other hand Gr II participants having LEAD accounted for 4.5% of all participants and 34.6% of the participants having LEAD. The prevalence of LEAD among participants of Gr I and Gr II was found to be 16.8% (95% CI 14.5-19.1) and 8.9% (95% CI 7.1-10.7) respectively. Moreover LEAD was found to be significantly higher (χ^2=27.776, df 1; p<0.001) amongst participants of Gr I than among participants of Gr II. It was also found that those having arsenicosis (Gr I) about 2.1 (1.6-2.7) times more likely (χ^2=28.175, df 1; p<0.001) to have LEAD than those not having arsenicosis and whose drinking water arsenic content was more than 0.05 mg/L (Gr II).

Amongst the total 835 male participants in Gr I and Gr II, 51.8% were members of Gr I. The overall prevalence of LEAD among these participants was found to be 12.2% (95% CI 10.0-14.4). Amongst the 102 participants having LEAD 69.6% were members of Gr I. The prevalence of LEAD among the Gr I and Gr II participants were 16.4% (95% CI CI 12.9-19.9) and 7.7% (95% CI 5.1-10.3) respectively. It was found that significantly higher number (χ^2= 14.666, df 1; p<0.05) of participants of Gr I had LEAD than participants of Gr II. Moreover it was found that males having arsenicosis (Gr I) had almost 2.3 times (95% CI 1.5-3.7) higher risk of having LEAD than males having arsenic exposure group (drinking water arsenic content >0.05mg/L) but not having arsenicosis (χ^2=15.075, df1; p<0.001).

Amongst the total 1164 female participants in Gr I and Gr II, 48.7% were members of Gr I. The overall prevalence of LEAD among these participants was found to be 13.3% (95% CI 11.4-15.2). Amongst the 155 participants having LEAD 62.6% were members of Gr I. The prevalence of LEAD among the Gr I and Gr II participants were 17.1% (95% CI 14.0-20.2) and 9.7% (95% CI 7.3-12.1) respectively. It was also found that significantly higher number (χ^2= 13.767, df 1; p<0.05) of participants of Gr I had LEAD than those of Gr II. Moreover it was found that those having arsenicosis had almost 1.9 (95% CI 1.4 to 2.7) times higher risk of having LEAD than those having arsenic exposure

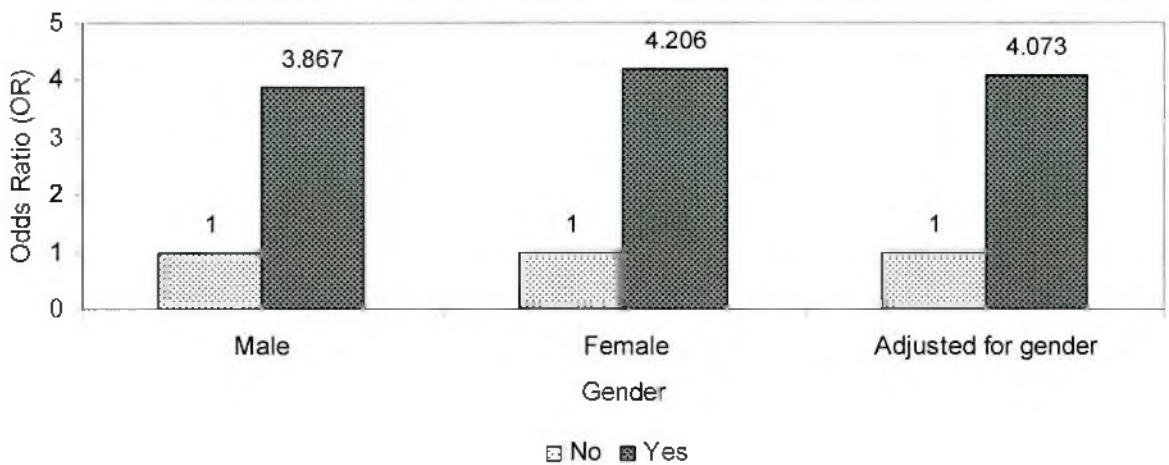
group (drinking water arsenic content >0.05mg/L) but not having arsenicosis (χ^2= 13.868, df1; p<0.001).

Finally when adjusted for gender LEAD was about 2.1 (1.6-2.7) times more likely (p<0.001) amongst those whose drinking water arsenic content was >0.05 mg/L and had arsenicosis, than those whose who had arsenic exposure (drinking water arsenic content >.05 mg/L) but had not developed arsenicosis (χ^2= 28.978, df 2; p<0.001).

Table-47: Prevalence of LEAD amongst study participants by excess arsenic exposure and gender.

Excess Arsenic exposure	Gender									OR (95% (CI)		
	Male			Female			Combined					
	Total	LEAD		Total	LEAD		Total	LEAD				
		Yes	Prevalence (%)		Yes	Prevalence (%)		Yes	Prevalence (%)	Male	Female	Adjusted for gender
Yes	835 (67.4)	102 (87.9)	12.2	1164 (66.1)	155 (88.1)	13.3	1999 (66.7)	257 (88.0)	12.9	3.867 (2.188- 6.868)	4.206 (2.636-6.711)	4.073 (2.837-5.849)
No	404 (32.6)	14 (12.1)	3.5	596 (33.9)	21 (11.9)	3.5	1000 (33.3)	35 (12.0)	3.5	1	1	1
Total	1239	116	9.4	1760	176	10.0	2999	292	9.7	χ^2= 28.713, df1; p<0.001	χ^2= 49.124, df1; p<0.001	χ^2= 78.127, df 2; p<0.001
Significance	χ^2= 24.566, df 1; p<0.001			χ^2= 42.0, df 1; p<0.001			χ^2= 66.395, df 1; <0.001					

Fig No-27: LEAD amongst study participants by excess arsenic exposure and gender.



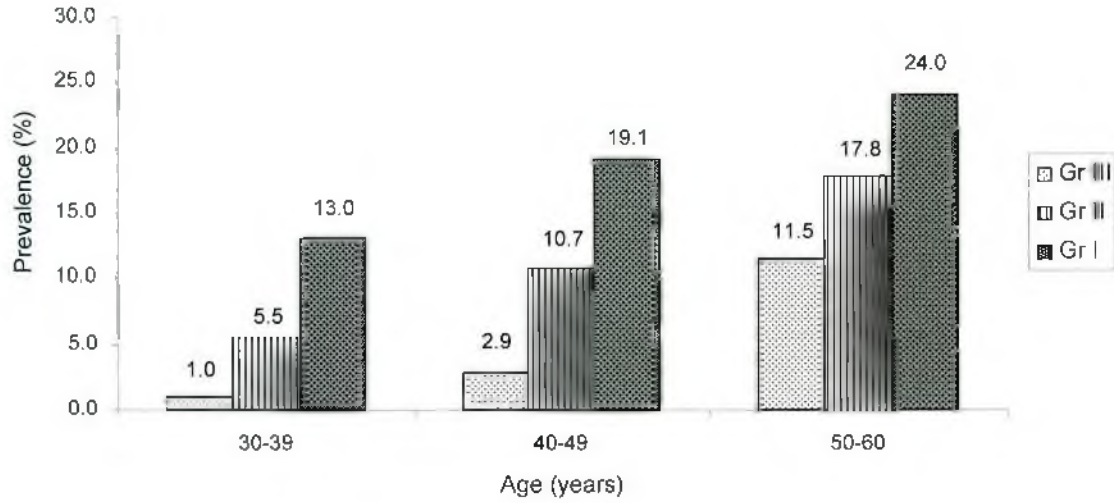
Amongst the total 2999 participants, 292 (9.7%) were found to have LEAD. Of them 257 had arsenic exposure (drinking water arsenic content >0.05 mg/L) and the other 35 were those who were considered as non-exposed (drinking water arsenic content <0.05 mg/L). Participants having LEAD among the exposed group accounted for 8.6% of all participants and 88.0% of all participants having LEAD. On the other hand participants having LEAD among the non exposed group accounted for 1.2% of all participants and 12.0% of the participants having LEAD. The prevalence of LEAD among participants exposed to excess arsenic and those having no such exposure was found to be 12.9% (95% CI 11.4-14.3) and 3.5% (95% CI 2.4-4.6) respectively. Moreover LEAD was found to be significantly higher (χ^2=66.395, df 1; p<0.001) amongst those having excess arsenic exposure than amongst those not having such exposure. It was also found that those having arsenic exposure (drinking water arsenic content >0.05 mg/L) were about 4.1 (2.8-5.8) times more likely to have LEAD than those having no such exposure (<0.05 mg/L) it was found to be statistically significant (χ^2= 77.644, df 1; p<0.05).

Among 1239 male participants of the study 67.4% could be grouped as having excess arsenic exposure (drinking water arsenic content >0.05mg/L) while the remaining 404 (32.6%) collected water from sources that yielded water having arsenic at levels lower than 0.05 mg/L. Of them 116 (9.4%) had LEAD, and amongst them 87.9% had excess arsenic exposure while the remaining 37.8% collected water from sources that yielded water contain arsenic at levels less than 0.05mg/L. The prevalence of arsenic amongst those whose drinking water arsenic content was >0.05mg/L (arsenic exposure) was found to be 12.2% (95% CI 10.0-14.4) while that amongst those having no such exposure (drinking water arsenic content <0.05mg/L) was found to be 3.5% (95% CI 1.7-5.2). The difference in LEAD between the groups was found to be statistically significant (χ^2= 24.512, df 1; p<0.05). More over it was also found that males having excess arsenic exposure (drinking water arsenic content was >0.05mg/L) was about 3.9 times (95% CI 2.2-6.9) more likely (χ^2= 28.713, df 1; p<0.001) to have LEAD than males having no excess arsenic exposure (drinking water arsenic content <0.05mg/L).

Among 1760 female participants of the study 66.1% could be grouped as having excess arsenic exposure (drinking water arsenic content >0.05mg/L) while the remaining 596 (33.9%) collected water from sources that yielded water having arsenic at levels lower than 0.05 mg/L. Of them 176 (10.0%) had LEAD, and amongst them 88.1% had excess arsenic exposure while the remaining 11.9% collected water from sources that yielded water contain arsenic at levels less than 0.05mg/L. The prevalence of arsenic amongst those whose drinking water arsenic content was >0.05mg/L (arsenic exposure) was found to be 13.3% (95% CI 11.4-15.3) while that amongst those having no such exposure (drinking water arsenic content <0.05mg/L) was found to be 3.5% (95% CI 2.0-5.0). The difference in LEAD between the groups was found to be statistically significant (χ^2= 42.0, df 1; p<0.001). More over it was also found that females having excess arsenic exposure (drinking water arsenic content was >0.05mg/L) was about 4.2 times (95% CI 2.3-6.7) more likely (χ^2= 49.124, df 1; p<0.001) to have LEAD than females having no excess arsenic exposure (drinking water arsenic content <0.05mg/L).

Finally when adjusted for gender LEAD was found to be about 4.1 (2.8-5.8) times more likely amongst those whose drinking water arsenic content was >0.05 mg/L (excess arsenic exposure) than those having no such excess arsenic exposure (χ^2= 78.127, df 2; p<0.001).

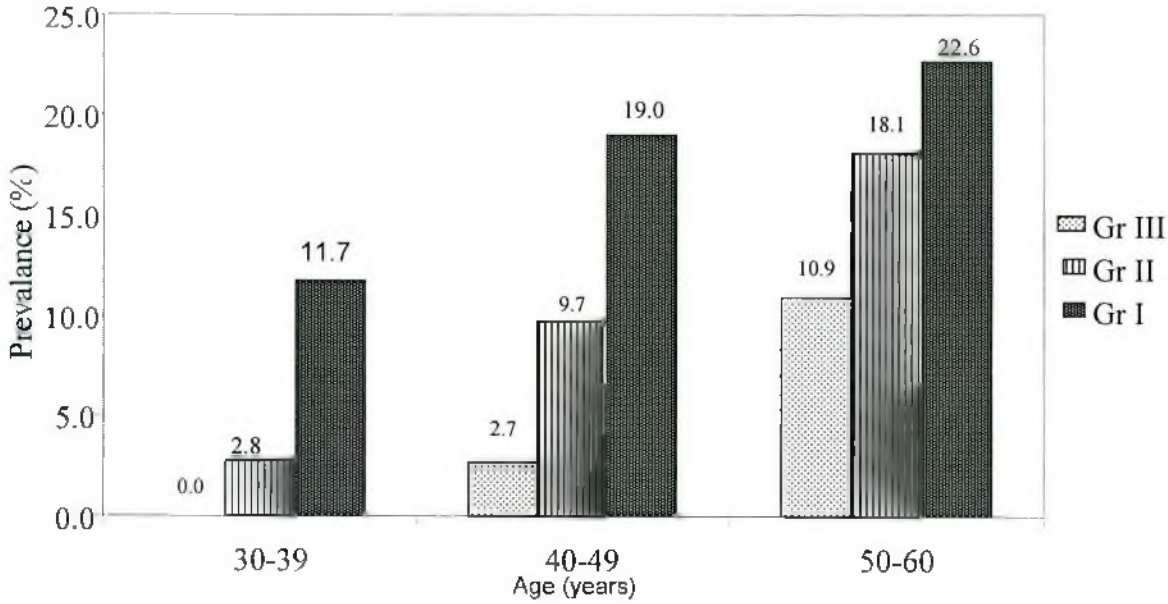
Figure -28: Prevalence of LEAD by age and study groups.



The prevalence of LEAD amongst participants aged 30-39 years amongst participants of Gr I, Gr II and Gr III was found to be 13.0%, 5.5% and 1.0% respectively. In all the study groups it was found that the prevalence had increased with age. And the highest prevalence was found amongst those who belonged to the age group of 50 to 60 years, and it was 24.0%, 17.8% and 11.5% for Gr I, Gr II and Gr III respectively.

Moreover a statistically significant association between age and LEAD was detected in all the study groups [(Gr I: χ^2= 12.136, df 2; p=0.002), (Gr II: χ^2=23.939, df 2; p <0.001) and (Gr III: χ^2=41.937, df 2; p <0.001)

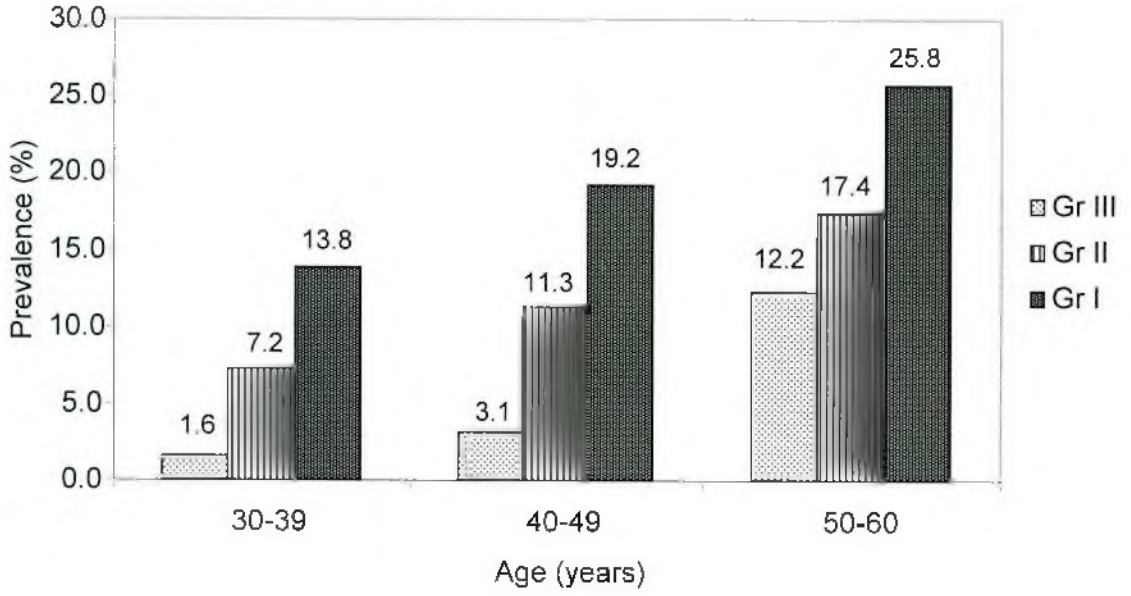
Figure -29: Prevalence of LEAD among male participants by age and study groups.



The prevalence of LEAD amongst male participants aged 30-39 years of Gr I, Gr II and Gr III was found to be 11.7%, 2.8% and 0.0% respectively. In all the study groups it was found that the prevalence had increased with age. And the highest prevalence was found amongst those who belonged to the age group of 55 to 60 years, and it was 22.6%, 18.1% and 10.9% for Gr I , Gr II and Gr III respectively.

Moreover a statistically significant association between age and LEAD was detected among males in all the study groups [(Gr I: χ^2= 6.21, df 2; p=0.045), (Gr II: χ^2= 20.484, df 2; p <0.001) and (Gr III: χ^2= 21.221, df 2; p <0.001)].

Figure -30: Prevalence of LEAD among female participants by age and study groups.



The prevalence of PAD amongst female participants aged 30-39 years of Gr I, Gr II and Gr III was found to be 13.8%, 7.2% and 1.6% respectively. In all the study groups it was found that the prevalence had increased with age. And the highest prevalence was found amongst those who belonged to the age group of 50 to 60 years, and it was 25.8%, 17.4% and 12.2% for Gr I, Gr II and Gr III respectively.

Moreover a statistically significant association between age and LEAD was detected among females in all the study groups [(Gr I: χ^2= 6.473, df 2; p=0.039), (Gr II: χ^2= 7.569, df 2; p <0.001) and (Gr III: χ^2= 21.824, df 2; p <0.001)].

Table -48: Distribution of participants by study groups, age, gender and LEAD.

Age Group (years)	Study Group	Gender									Significance		
		Male			Female			Combined					
		Total	LEAD		Total	LEAD		Total	LEAD		Male	Female	Combined
			Yes	Prevalence (%)		Yes	Prevalence (%)		Yes	Prevalence (%)			
30-39	Gr I	196	23	11.7	298	41	13.8	494	64	13.0	χ^2= 29.398, df 2; p<0.001	χ^2 = 33.459, df 2; p<0.001	χ^2 = 58.577, df 2; p<0.001
	Gr II	216	6	2.8	333	24	7.2	549	30	5.5			
	Gr III	164	0	0.0	318	5	1.6	482	5	1.0			
40-49	Gr I	153	29	19.0	203	39	19.2	356	68	19.1	χ^2= 21.018, df 2; p<0.001	χ^2 = 25.992, df 2; p<0.001	χ^2 = 46.817, df 2; p<0.001
	Gr II	103	10	9.7	195	22	11.3	298	32	10.7			
	Gr III	148	4	2.7	196	6	3.1	344	10	2.9			
50-60	Gr I	84	19	22.6	66	17	25.8	150	36	24.0	χ^2= 4.400, df 2; p=0.111	χ^2 = 4.586, df 2; p=0.101	χ^2 = 8.768, df 2; p=0.012
	Gr II	83	15	18.1	69	12	17.4	152	27	17.8			
	Gr III	92	10	10.9	82	10	12.2	174	20	11.9			
All ages	Gr I	433	71	16.4	567	97	17.1	1000	168	16.8	χ^2= 43.099, df 2; p<0.001	χ^2 = 59.657, df 2; p=0.012	χ^2 =101.804, df 2; p<0.001
	Gr II	402	31	8.3	597	58	9.7	999	89	8.9			
	Gr III	404	14	3.5	596	21	3.5	1000	35	3.5			
	Total	1239	116	9.4	1760	176	10.0	2999	292	9.7			
Age adjusted prevalence	Gr I	16.4% (14.3-18.4)			17.1%(15.3- 18.8)			16.8% (15.4-18.1)					
	Gr II	8.2% (6.7-9.7)			9.8% (8.4-11.2)			9.2% (8.1-10.2)					
	Gr III	3.2% (2.2-4.2)			3.4% (2.5-4.2)			3.3% (2.7-4.0)					
OR (95% CI) Adjusted for age	Gr I	6.066 (3.335-11.032)			5.885 (3.602-9.615)			5.900 (4.038-8.620)			χ^2=77.711, df 4; p<0.001	χ^2=86.600, df 4; p<0.001	χ^2=159.476, df 4; p<0.001
	Gr II	2.629 (1.366-5.057)			3.074 (1.835-5.149)			2.885 (1.925-4.323)					
	Gr III	1			1			1					
Age and gender adjusted prevalence	Gr I	16.8 (15.448-18.123)											
	Gr II	9.2 (8.143-10.210)											
	Gr III	3.3 (2.650-3.926)											
OR (95% CI) Adjusted for age & gender	Gr I	5.946 (4.068-8.690)									χ^2= 162.351, df 5; p<0.001		
	Gr II	2.883 (1.924-4.320)											
	Gr III	1											

Among the 2999 study participants the proportion of respondents in the age stratum (years) 30-39, 40-49 and 50-60 were 50.8% (1525), 33.3% (998), and 15.9% (476) respectively. There were fewer participants in higher age strata in contrast to the lower age strata. The proportion of participants between the groups in each of the age strata varied from 31.4% to 36.5%, except for the stratum of 40-49 years in which the participants of Gr I, Gr II and Gr III accounted for 35.7%, 29.9% and 34.5% of the 998 participants of the stratum. In each of the age stratum the prevalence of LEAD was highest amongst those who had arsenicosis (Gr I) followed by those having arsenic exposure (drinking water arsenic content <0.05mg/L) but no signs of arsenicosis (Gr II). Participants whose drinking water arsenic content was less than 0.05 mg/L (Gr III) in each of the stratum had the lowest prevalence. Moreover the difference in the prevalence of LEAD between the groups was found to be statistically significant ($p<0.05$).

The crude prevalence rates of LEAD for participants of Gr I, Gr II or Gr III was 16.8% (14.5-19.1), 8.9% (7.1-10.7) and 3.5% (2.4-4.6) respectively. When adjusted for age the prevalence for Gr I remained unchanged at 16.8% (16.0-18.7), in Gr II it increased from 8.9% to 9.2% (8.1-10.2), on the other hand the prevalence for participants in Gr I had decreased from 3.5 to 3.3% (2.7-4.0). When adjusted for age, LEAD was still found to be 5.9 times (4.0-8.6) higher amongst those having arsenicosis (Gr I), and about 2.9 (1.9-4.3) times higher amongst those having arsenic exposure group (drinking water arsenic content >0.05mg/L) but not having arsenicosis (Gr II), than amongst those who collected their drinking water from source(s) that yielded water containing arsenic at levels less <0.05 mg/L (Gr III), (χ^2=159.476, df 4; $p<0.001$).

Amongst male, the proportion of respondents in the age stratum (years) 30-39, 40-49, and 50-60 were 46.5%, 32.6% and 20.9% respectively. There were fewer participants in higher age strata in contrast to the lower age strata. In each of the age stratum the prevalence of LEAD was highest amongst those who had arsenicosis (Gr I) followed by those having arsenic exposure (drinking water arsenic content <0.05mg/L) but no signs of arsenicosis (Gr II). Participants whose drinking water arsenic content was less than 0.05 mg/L (Gr III) in each of the stratum had the lowest prevalence. Moreover the difference in the prevalence of LEAD between the groups was found to be statistically significant ($p<0.05$) except for the age stratum 30-39 and 40-49 years. Though the

difference of LEAD between the groups in the age stratum 50-60 years was not significantly different, highest proportion of participants of Gr I had LEAD while it was lowest amongst participants of Gr III. The crude prevalence rates of LEAD for participants of Gr I, Gr II and Gr III was 16.4% (12.9-19.9), 8.5% (95% CI 7.1-10.7) and 3.5% (95% CI 2.4-4.6) respectively. When adjusted for age the prevalence for Gr II increased from 7.7% (5.1-10.3) to 8.2% (6.7-9.7). On the other hand it had decreased from 16.4% (12.9-19.9) to 16.3% (14.3-18.4) for Gr I and from 3.5% (1.7-5.3) to 3.2% (2.2-4.2) for group III. When adjusted for age, LEAD was still found to be 6.1 times (3.3-11.0) higher amongst those having arsenicosis (Gr I), and about 2.6 (1.4-5.1) times higher amongst those having arsenic exposure group (drinking water arsenic content >0.05mg/L) but not having arsenicosis (Gr II), than amongst those who collected their drinking water from source(s) that yielded water containing arsenic at levels less <0.05 mg/L (χ^2= 77.711, df 4; p<0.001).

Among the female participants the proportion of respondents in the age stratum (years) 30-39, 40-49, and 50-60 were 53.9%, 33.8% and 12.3% respectively. There were fewer participants in higher age strata in contrast to the lower age strata. In each of the age stratum the prevalence of LEAD was highest amongst those who had arsenicosis (Gr I) followed by those having arsenic exposure (drinking water arsenic content >0.05mg/L) but no signs of arsenicosis (Gr II). Participants whose drinking water arsenic content was less than 0.05 mg/L (Gr III) in each of the stratum had the lowest prevalence. Moreover the difference in the prevalence of LEAD between the groups was found to be statistically significant (p<0.05) except for the age stratum 30-39 and 40-49 years. Though the difference of LEAD between the groups in the age stratum 50-60 years was not significantly different, highest proportion of participants of Gr I had LEAD while it was lowest amongst participants of Gr III. The crude prevalence rates of LEAD for participants of Gr I, Gr II and Gr III was 17.1% (14.0-20.2), 9.7 % (7.3-12.1) and 3.5% (2.4-4.6) respectively. When adjusted for age, the prevalence remained unchanged for Gr I, increased from 9.7 % (7.3-12.1) to 9.8% (8.4-11.2) for Gr II and had decreased from 16.4% (12.9-19.9) to 16.3% (14.3-18.4) for Gr I and from 3.5% (1.7-5.3) to 3.4% (2.5-4.2) for group III. Among the female participants when adjusted for age, LEAD was

found to be 5.9 (3.6-9.6) times amongst those having arsenicosis, and 3.1 (1.8-5.1) times higher amongst those having excess arsenic exposure (>0.05 mg/L) but no having arsenicosis than amongst those not having arsenicosis and whose drinking water arsenic content was less than 0.05 mg/L (χ^2= 86.600, df 4; p<0.001).

Among the participants of age groups of 30-39 and 40-49 years whether male or female, the prevalence of LEAD was found to be highest amongst participants of Gr I followed by that for participants of Gr II and Gr III. And this observed difference in the prevalence of LEAD was found to be statistically significant (p<0.001). On the other hand among the participants aged 50-60 years whether male or female though the prevalence of LEAD showed a pattern similar to that found in the other age groups the difference between the study groups was not found to be significant (p>0.05).

When adjusted for age and gender the prevalence remained unchanged for Gr I, decreased from 9.7 % (7.3-12.1) to 9.2% (8.1-10.2), for Gr II and had decreased from 16.4% (12.9-19.9) to 16.3% (14.3-18.4) for Gr I and from 3.5% (1.7-5.3) to 3.3% (2.6-3.9) for group III.

Logistic regression revealed that when controlled for age and gender LEAD was about 5.9 (4.1-8.7) times more likely amongst those whose drinking water arsenic content was > 0.05 mg/L and who had arsenicosis (Gr I), and about 2.9 (1.9-4.3) times more likely to occur amongst those who have arsenic exposure (drinking water arsenic content >0.05 mg/L) but had not developed arsenicosis (Gr II), compared to those whose drinking water arsenic content was <0.05 mg/L (Gr III), (χ^2= 162.351, df 5; p<0.001).

Table-49: Prevalence and ORs of LEAD among participants by study groups (Gr I & II), adjusted for age, and gender.

Age Group (years)	Study group	Gender									Significance		
		Male			Female			Combined					
		Total	LEAD		Total	LEAD		Total	LEAD		Male	Female	Combined
			Yes	Prevalence (%)		Yes	Prevalence (%)		Yes	Prevalence (%)			
30-39	Gr I	196	23	11.7	298	41	13.8	494	64	13.0	χ^2= 12.559, df 1; p<0.001	χ^2= 7.305, df 1; p=0.007	χ^2= 17.79, df 1; p<0.001
	Gr II	216	6	2.8	333	24	7.2	549	30	5.5			
40-49	Gr I	153	29	19.0	203	39	19.2	356	68	19.1	χ^2= 4.075, df 1; p=0.044	χ^2= 4.819, df 1; p<0.028	χ^2= 8.75, df 1; p=0.003
	Gr II	103	10	9.7	195	22	11.3	298	32	10.7			
50-60	Gr I	84	19	22.6	66	17	25.8	150	36	24.0	χ^2= 0.532, df 1; p=0.466	χ^2= 1.4, df 1; p=0.237	χ^2= 1.85, df 1; p= 0.173
	Gr II	83	15	18.1	69	12	17.4	152	27	17.8			
All ages	Gr I	433	71	16.4	567	97	17.1	1000	168	16.8			
	Gr II	402	31	8.3	597	58	9.7	999	89	8.9			
	Total	835	102	12.2	1164	155	13.3	1999	257	12.9			
Age adjusted prevalence	Gr I	16.1 (13.630-18.619)			17.0 (14.856-19.173)			16.6 (15.002-18.267)					
	Gr II	8.0 (6.124-9.796)			9.8 (8.075-11.488)			9.0 (7.790-10.305)					
OR (95% CI) adjusted for age	Gr I	2.279 (1.448-3.587)			1.906 (1.343-2.705)			2.032 (1.541-2.678)			χ^2= 36.312, df 3; p<0.001	χ^2= 26.857, df 3; p<0.001	χ^2= 59.754, df 3; p<0.001
	Gr II	1			1			1					
Age and gender adjusted prevalence	Gr I	16.6 (15.010-18.276)											
	Gr II	9.0 (7.765-10.277)											
OR (95% CI) adjusted for age and gender	Gr I	2.048 (1.553-2.700)									χ^2= 61.870, df 4; p<0.001		
	Gr II	1											

Amongst the 1999 participants of Gr I and Gr II a total of 257 (12.9%) participants were found to have LEAD. In the age strata 30-39 and 40-49 significantly higher proportions of participants in Gr I were found to have LEAD than in participants of GR II ($p<0.05$). The prevalence of LEAD among Gr I participants were 13.0%, and 19.1% for those in the age stratum of 30-39, and 40-49 years respectively, on the other hand 5.5% and 10.7% participants of Gr II belonging to the age stratum of 30-39 and 40-49 years respectively were found to have LEAD. But in age strata 50-60 years though the prevalence of LEAD was found to be higher amongst participants of Gr I (24.0%) in contrast to participants of Gr II (17.8%), the difference was not statistically significant ($p>0.05$). Moreover LEAD was found to be 2.6 (1.6-4.0), 2.0 (1.2-3.1) and 1.5 (0.8-2.6) times higher in the age stratum of 30-39, 40-49 and 50-60 years respectively, amongst participants of Gr I than among participants of Gr II. When adjusted for age, LEAD was found to be about 2.0 (1.5-2.7) times higher amongst those having arsenic exposure group (drinking water arsenic content >0.05mg/L) and arsenicosis, than amongst those who collected their drinking water from source(s) that yielded water containing arsenic at levels >0.05 mg/L but not having arsenicosis ($\chi^2= 59.754$, df 3; $p<0.001$).

Amongst the 835 male participants 51.8% (433) were members of Gr I and 48.2% (402) belonged to Gr II. The overall prevalence of LEAD among the male participants of Gr I and Gr II was 12.2%. The prevalence of LEAD among participants of Gr I and Gr II was found to be 16.4% and 8.0% respectively. In the age strata 30-39 and 40-49 years LEAD was found to be significantly higher ($p<0.05$) amongst those having arsenicosis and exposure to excess arsenic (Gr I) than amongst those having arsenic exposure group (drinking water arsenic content >0.05mg/L) but no arsenicosis (Gr II). In the stratum of 50-60 years though LEAD was more prevalent amongst the members of Gr I than amongst members of Gr II, the difference was not statistically significant ($p>0.05$). When adjusted for age, LEAD was found to be 2.3 (1.4-3.6) higher amongst those having arsenicosis and excess arsenic exposure than amongst those whose drinking water arsenic content was more than 0.05 mg/L but did not have arsenicosis ($\chi^2= 36.312$, df 3; $p<0.001$).

Amongst the 1164 female participants 48.7% (567) were members of Gr I and 51.3% (597) belonged to Gr II. The overall prevalence of LEAD among the female participants of Gr I and Gr II was 13.3%. The prevalence of LEAD among participants of Gr I and Gr II was found to be 17.1% and 9.7% respectively. In the age strata 30-39 and 40-49 years LEAD was found to be significantly higher ($p<0.05$) amongst those having arsenicosis and exposure to excess arsenic (Gr I) than amongst those having arsenic exposure group (drinking water arsenic content >0.05mg/L) but no arsenicosis (Gr II). Whereas in the stratum of 50-60 years though LEAD was more prevalent amongst the female members of Gr II than amongst female members of Gr II, the difference was not statistically significant ($p>0.05$). When adjusted for age, LEAD was found to be 1.9 (1.3-2.7) higher amongst those having arsenicosis and excess arsenic exposure, than amongst those whose drinking water arsenic content was >0.05 mg/L but did not have arsenicosis ($\chi^2= 26.857$, df 3; $p<0.001$).

Finally when adjusted for age, and gender LEAD was found to be about 2.0 (1.5-2.7) times more prevalent ($\chi^2= 61.870$, df 4; $p<0.001$) amongst those having excess arsenic exposure participants than amongst participants not having such exposure.

Table-50 : Prevalence's, and ORs of LEAD among participants by study groups (Gr I & II), adjusted for age, and gender.

Age Group (years)	Excess Arsenic exposure	Gender									Significance		
		Male			Female			Combined					
		Total	LEAD		Total	LEAD		Total	LEAD		Male	Female	Combined
			Yes	Prevalence (%)		Yes	Prevalence (%)		Yes	Prevalence (%)			
30-39	Yes	412	29	7.0	631	65	10.3	1043	94	9.0	χ^2= 12.156, df 1; p<0.0001	χ^2= 23.580, df 1; P<0.0001	χ^2= 34.54, df 1; p<0.0001
	No	164	0	0.0	318	5	1.6	482	5	1.0			
40-49	Yes	256	39	15.2	398	61	15.3	654	100	15.3	χ^2= 15.486, df 1; p<0.0001	χ^2= 19.743, df 1; p<0.0001	χ^2= 35.24, df 1; p<0.0001
	No	148	4	2.7	196	6	3.1	344	10	2.9			
50-60	Yes	167	34	20.4	135	29	21.5	302	63	20.9	χ^2= 3.738, df 1; p=0.052	χ^2= 2.984, df 1; p=0.084	χ^2= 6.73, df 1; p= 0.009
	No	92	10	10.9	82	10	10.2	174	20	11.5			
All ages	Yes	835	102	12.2	1164	155	13.3	1999	257	12.9			
	No	404	14	3.5	596	21	3.5	1000	35	3.5			
	Total	1239	116	9.4	1760	176	10.0	2999	292	9.7			
Age adjusted prevalence	Yes	12.5 (10.654-14.337)			13.4 (11.785-14.966)			13.0 (11.778-14.184)					
	No	3.2 (2.180-4.127)			3.4 (2.540-4.229)			3.3 (2.677-3.959)					
OR (95% CI) Adjusted for age	Yes	4.353 (2.443-7.758)			4.382 (2.739-7.012)			4.333 (3.009-6.238)			χ^2= 63.927, df 3; p<0.001	χ^2= 73.093, df 3; p<0.001	χ^2= 132.822, df 3; p<0.001
	No	1			1			1					
Age and gender adjusted prevalence	Yes	13.5 (12.255-14.700)											
	No	3.3 (2.651-3.927)											
OR (95% CI) Adjusted for age and gender	Yes	4.345 (3.018- 6.257)									χ^2= 135.113, df 4; p<0.001		
	No	1											

Amongst the 2999 study participants, 1999 (66.7%) had excess arsenic exposure group (drinking water arsenic content >0.05mg/L). The overall prevalence of LEAD as found to be 9.7% (292). The proportion of participants in the age strata 30-39, 40-49 and 50-60 the proportion of participants having excess arsenic exposure was found to be 68.4%, 65.5% and 63.4% respectively. In each of the age strata, prevalence of LEAD was found to be significantly higher ($p<0.05$) amongst those having excess arsenic exposure. LEAD was found to be 9.5, 6.0 and 2.2 times higher amongst those having excess exposure than those having no such exposure in the age stratum of 30-39, 40-49 and 50-60 years respectively. When adjusted for age, LEAD was still found to be about 4.333 (3.009-6.238) times higher amongst those having excess arsenic exposure group (drinking water arsenic content >0.05mg/L) than amongst those who collected their drinking water from source(s) that yielded water containing arsenic at levels less than 0.05 mg/L ($\chi^2=132.822$, df 3; $p<0.001$).

Amongst the 1239 male study participants of 67.4% (835) had excess arsenic exposure group (drinking water arsenic content >0.05mg/L). The overall prevalence of LEAD among these male participants was found to be 9.4% (116). The prevalence of LEAD among participants having excess exposure and not having such exposure was found to be 12.2% and 3.5% respectively. In the age stratum of 30-39 and 40-49 years LEAD was found to be significantly higher ($p<0.05$) amongst participants having exposure to excess arsenic than among participants having no such exposure. On the other hand, in the age strata of 50-60 years though LEAD was more prevalent amongst those having exposure to excess arsenic than amongst those having no such exposure, the difference was marginally insignificant ($p=0.052$).When adjusted for age, LEAD was found to be about 4.3 (2.4-7.7) times higher amongst males having excess arsenic exposure (>0.05mg/L) group than amongst male participants whose drinking water from source(s) that yielded water containing arsenic at levels less than 0.05 mg/L ($\chi^2=63.927$, df 3; $p<0.001$).

Amongst the 1760 female study participants of 66.1% (1164) had excess arsenic exposure group (drinking water arsenic content >0.05mg/L). The overall prevalence of LEAD among these female participants was found to be 10.0% (176). The prevalence of LEAD among participants having excess exposure and not having such exposure was

found to be 13.3% and 3.5% respectively. In the age stratum of 30-39 and 40-49 years LEAD was found to be significantly higher ($p<0.05$) amongst participants having exposure to excess arsenic than among participants having no such exposure. On the other hand, in the age strata of 50-60 years though LEAD was more prevalent amongst those having exposure to excess arsenic than amongst those having no such exposure the difference was not statistically significant ($p=0.084$). When adjusted for age, LEAD was found to be about 4.4 (2.7-7.0) times higher amongst females having excess arsenic exposure (>0.05mg/L) group than amongst female participants who drinking water from source(s) that yielded water containing arsenic at levels less than 0.05 mg/L ($\chi^2=73.093$, df 3; $p<0.001$).

Finally when adjusted for age, and gender LEAD was still found to be 4.3 (3.0-6.2) times more prevalent ($\chi^2=135.113$, df 4; $p<0.001$) amongst those having excess arsenic exposure (>0.05mg/L) than amongst participants not having such exposure.

Table-51: Odds ratio and their 95% CI of LEAD among the study groups adjusted for different socio-demographic variables

Gender	Combined			Male			Female		
Study Groups	Gr III	Gr II	Gr I	Gr III	Gr II	Gr I	Gr III	Gr II	Gr I
Crude OR	1	2.697 (1.805-4.028)	5.567 (3.823-8.107)	1	2.328 (1.219-4.445)	5.464 (3.026-9.864)	1	2.946 (1.764-4.920)	5.651 (3.472-9.199)
ORs adjusted for Socio-demographic variables									
Adjusted for age	1	2.572 (1.337-4.949)	6.052 (4.138-8.851)	1	2.629 (1.366-5.057)	6.335 (3.473-11.556)	1	3.062 (1.827-5.130)	5.954 (3.642-9.73[illegible])
Adjusted for gender	1	2.696 (1.805-4.028	5.587 (3.836-8.136)						
Adjusted for age & gender	1	2.883 (1.924-4.320)	5.946 (4.068-8.690)						
Adjusted for household size	1	2.676 (1.791-3.999)	5.533 (3.799-8.058)	1	2.281 (1.193-4.362)	5.552 (3.0730-10.031)	1	2.952 (1.767-4.931)	5.669 (3.479-9.23[illegible])
Adjusted for education level	1	2.704 (1.810-4.042)	5.593 (3.840-8.146)	1	2.356 (1.233-4.503)	5.533 (3.062-9.997)	1	3.007 (1.797-5.031)	5.751 (3.528-9.37[illegible])
Adjusted for occupation	1	2.728 (1.825-4.077)	5.607 (3.849-8.167)	1	2.385 (1.244-4.574)	5.594 (3.086-10.140)	1	3.001 (1.794-5.018)	5.678 (3.484-9.25[illegible])
Adjusted for household possession of agricultural land	1	2.763 (1.848-4.132)	5.597 (3.843-8.153)	1	2.363 (1.236-4.516)	5.506 (3.048-9.947)	1	3.111 (1.858-5.211)	5.745 (3.526-9.360)
Adjusted for housing type	1	2.680 (1.793-4.005)	5.543 (3.806-8.073)	1	2.318 (1.212-4.433)	5.414 (2.991-9.800)	1	2.949 (1.765-4.925)	5.688 (3.492-9.264)
Adjusted for Tv ownership	1	2.695 (1.804-4.026)	5.462 (3.749-7.959)	1	2.321 (1.215-4.433)	5.397 (2.983-9.765)	1	2.963 (1.774-4.951)	5.557 (3.412-9.050)
Adjusted for radio ownership	1	2.812 (1.878-4.212)	5.750 (3.942-8.388)	1	2.490 (1.296-4.784)	5.757 (3.174-10.442)	1	3.030 (1.811-5.071)	5.772 (3.541-9.408)
Adjusted for bicycle ownership	1	2.717 (1.814-4.071)	5.605 (3.840-8.181)	1	2.214 (1.153-4.250)	5.244 (2.894-9.502)	1	3.085 (1.840-5.172)	5.907 (3.615-9.654)
Adjusted for motorcycle ownership	1	2.724 (1.822- 4.072)	5.576 (3.829-8.120)	1	2.328 (1.219-4.445)	5.468 (3.028-9.875)	1	3.031 (1.811-5.073)	5.715 (3.509-9.309)
Adjusted for annual household income	1	2.697 (1.805-4.029)	5.567 (3.823-8.106)	1	2.359 (1.233-4.515)	5.551 (3.065-10.055)	1	2.993 (1.789-5.006)	5.739 (3.519-9.359)
All socio-demographic variables	1	3.088 (2.037-4.682)	6.263 (4.240-9.251)	1	2.619 (1.324-5.181)	6.770 (3.610-12.698)	1	3.462 (2.029-5.908)	6.111 (3.682-10.141)

LEAD was found to be more prevalent among the members of Gr I (5.6 times) followed by that for members of Gr II (2.7 times) compared to members of Gr III (χ^2=105.818, df 2; p<0.001). The crude ORs for LEAD among males in Gr II and Gr I were 2.3 and 5.5 respectively (χ^2=43.787, df 2; p<0.001), and among females it was 2.9 and 5.6 respectively in reference to that for members of Gr III (χ^2=62.992, df 2; p<0.001).

OR and their 95% confidence intervals for LEAD in the different study groups adjusted for individual and all socio-demographic variables together were obtained by logistic regression.

When adjusted for age LEAD was observed to be 6.0 times and 2.9 times more common among members of Gr I and Gr II respectively than among members of Gr III (χ^2=168.973, df 3; p<0.001). In males LEAD was 6.3 times and 2.6 times more common among members of Gr I and Gr II respectively than among members of Gr III (χ^2=82.776, df 3; p<0.001). On the other hand among females when adjusted for age LEAD was found to be 5.9 times and 3.1 times more common among members of Gr I and Gr II respectively than among members of Gr III (χ^2=90.906, df 3; p<0.001).

When adjusted for gender LEAD was found to be 2.7 times and 5.6 times higher among members of Gr II and Gr I respectively than among members of Gr III (χ^2=106.546, df 3; p<0.001). Both in the case of age and gender the adjusted ORs were found to be higher than the crude ORs.

As for the other socio-demographic variables the adjusted ORs for LEAD among members of Gr I and Gr II were generally almost same or higher than the crude ORs .

When adjusted for socio-demographic variables (age, household size, education level, occupation, housing type, annual household income, and household possession of agricultural land, radio, bicycle, television and motorcycle) excluding gender LEAD was found to be 6.1 (4.2-9.2) times and 3.5(2.0-5.9) times more prevalent among female members of Gr I and Gr II respectively than among female members of Gr III (χ^2=114.289, df 16; p<0.001), and 6.8 (3.6-12.7) times and 2.6 (1.3-5.2) times more prevalent among male members of Gr I and Gr II respectively than among male members of Gr III (χ^2=107.393, df 17; p<0.001).

When adjusted for all socio-demographic variables (age, gender, household size, education level, occupation, housing type, annual household income, and household

possession of agricultural land, radio, bicycle, television and motorcycle) LEAD was found to be 6.3 (4.2-9.2) times and 3.1(2.0-4.7) times more prevalent among members of Gr I and Gr II respectively than among members of Gr III (χ^2= 194.228, df 18; p<0.001).

4.3 FOOD HABIT & TOBACOO USE RELATED VARIABLES AND LEAD BY STUDY GROUPS

Table-52: Distribution of participants by staple food type, study groups and LEAD.

Staple food type	Study group	Total	LEAD		Significance	OR (95% CI)
			Yes	Prevalence (%)		
Starch based	Gr I	466	65	13.9	χ^2= 13.260, df 2; p=0.001	4.944 (1.765-13.846)
	Gr II	462	44	9.5		3.211 (1.131-9.113)
	Gr III	126	4	3.2		1
Starch & Vegetable protein based	Gr I	534	103	19.3	χ^2= 98.961, df 2; p<0.001	6.499 (4.279-9.871)
	Gr II	537	45	8.4		2.487 (1.553-3.983)
	Gr III	874	31	3.5		1
Total		2999	292	9.7		
OR (95% CI) adjusted for staple food type	Gr I	5.926 (4.031-8.711)			χ^2= 107.841, df 3; p<0.001	
	Gr II	2.866 (1.903-4.318)				
	Gr III	1				

Out of the 2999 participants of all three study groups 35.1% (1054) consumed starch based staple food, the other 64.9% (1766) consumed starch and vegetable protein based staple food. Whatever the type of staple food consumed LEAD was found to be a significantly (p<0.05) higher amongst participants of Gr I and Gr II than among participants of Gr III. Amongst those who consumed starch based staple food, LEAD was found to be 4.9 (1.8-13.4) times and 3.2 (1.1-9.1) times more prevalent among members of Gr I and Gr II respectively than among members of Gr III (χ^2= 15.460, df 2; p<0.001). Similarly LEAD was observed to be about 6.5 (4.3-9.9) times and 2.5 (1.5-4.0) times more prevalent among members of Gr I and Gr II respectively than among members of Gr III (χ^2= 94.159, df 2; p<0.001) who consumed staple food that was starch and vegetable protein based.

Finally when adjusted for staple food type LEAD was found to be about 5.9 (4.0-8.7) times and 2.9 (1.9-4.3) more prevalent (χ^2= 107.841, df 3; p<0.001) amongst participants of Gr I and Gr II participants respectively than amongst participants of Gr III.

Table- 53: Distribution of LEAD among participants of groups I and II by staple food type.

Staple food type	Study group		LEAD		Significance OR (95% CI)
		Total	Yes	Prevalence (%)	
Starch based (928)	Gr I	466	65	13.9	1.540 (1.026-2.312) χ^2= 4.407, df 1; p=0.036
	Gr II	462	44	9.5	
Starch & Vegetable protein (1071)	Gr I	534	103	19.3	2.613 (1.799-3.795) χ^2= 27.376, df 1; p<0.001
	Gr II	537	45	8.4	
Total		1999	257	12.9	
Adjusted for type of staple food consumed	Gr I	2.067 (1.572-2.719)			χ^2= 30.170, df 2; p<0.001
	Gr II	1			

Out of the 1999 participants of Gr I and II 46.2% (928) consumed starch based staple food the other 53.8% consumed starch and vegetable protein based staple food. Whatever the type of staple food was consumed LEAD was found to be a significantly ($p<0.05$) common amongst participants of Gr I than among participants of Gr II. Amongst participants consuming starch based staple food LEAD was found to be 1.5 times higher amongst participants of Gr I compared to that amongst participants of Gr II (χ^2= 4.407, df 1; p=0.036). On the other hand, amongst participants consuming starch and vegetable protein based staple food; LEAD was found to be about 2.6 times higher amongst participants of Gr I compared to that amongst participants of Gr II (χ^2= 27.376, df 1; $p<0.001$). Finally when adjusted for the type of staple food consumed it was also found that those having arsenicosis were about 2.1 times more likely to have LEAD than those not having arsenicosis and whose drinking water arsenic content was more than 0.05 mg/L (χ^2= 30.170, df 2; $p<0.001$).

Table-54: Distribution of participants by excess arsenic exposure, staple food type and LEAD

Staple food type	Excess Arsenic exposure	Total	LEAD		Significance
			Yes	Prevalence (%)	
Starch based (1054)	Yes	928	109	11.7	χ^2= 8.515, df 1; p=0.004
	No	126	4	3.2	
Starch & Veg protein based (1945)	Yes	1071	148	13.8	χ^2= 60.769, df 1; p<0.001
	No	874	31	3.5	
OR (95% CI) Adjusted for staple food type	Yes	4.32 (2.98-6.262)			χ^2=79.589, df 2; p<0.001
	No	1			

Out of the total 2999 study participants 66.7% (1999) had excess arsenic exposure. Among them 9.7% (292) were found to have LEAD. About 35.1% (1054) participants mentioned that their staple food included either rice or rice and potato (starch based). Among those whose staple food was starch and vegetable protein based (rice and pulses), 9.2% (179) was found to have LEAD. On the other hand, among those whose staple food was starch based 10.7% (113) had LEAD. Amongst participants whose staple food was starch based, the prevalence of LEAD was found to be 11.7% if they had excess arsenic exposure and 3.2% if they had no such exposure. Again amongst participants whose staple food was starch and vegetable protein based the prevalence of LEAD was 13.8% if they had excess arsenic exposure on the other hand this prevalence was 3.5% among those having no such exposure. And the difference in the prevalence of LEAD between those having excess arsenic exposure and those having no such exposure was significantly different ($p<0.05$) in participants grouped by staple food type.

Moreover when adjusted for staple food type LEAD was found to be about 4.3 (3.0-6.3) times more prevalent (χ^2= 79.589, df 2; $p<0.001$) amongst participants having excess arsenic exposure than those having no such exposure.

Table-55: Distribution of LEAD among study participants by days of consumption of vegetable, study groups and LEAD.

Study group	Vegetables consumed per week						OR (95% CI) adjusted for vegetables consumed per week
	<7 days a week			7 days a week			
	Total	LEAD		Total	LEAD		
		Yes	Prevalence (%)		Yes	Prevalence (%)	
Gr I	40	6	15.0	960	162	16.9	5.612 (3.851-8.177)
Gr II	72	5	6.9	927	84	9.1	2.735 (1.827 4.094)
Gr III	0	0	0	1000	35	3.5	1
Total	112	11	9.8	2887	281	9.7	χ^2= 106.261, df 2; p<0.001
Significance	Fisher's Exact value=0.196			χ^2= 100.429, df 2; p<0.001			

Amongst the 2999 study participants only about 10.7% (112) consumed vegetables less than 7 days a week. The prevalence of LEAD amongst those who consumed vegetables less than 7 days a week was found to be 9.8% whereas that amongst those who consumed vegetables 7 days a week was found to be 9.7 %. And this observed difference in the prevalence between the vegetable consumption groups was not statistically significant (p=0.975). Amongst the 112 participants who consumed vegetables for less than 7 days per week none were members of Gr III, and the prevalence of LEAD was higher amongst members of Gr I (15.0%) than amongst members of Gr II (6.9%).

Amongst the 2887 participants who consumed vegetables for 7 days per week the prevalence of LEAD was higher amongst members of Gr I, Gr II and Gr III were 16.9%, 9.1% and 3.5% respectively. And the difference in prevalence between the groups was found to be statistically significant (p<0.05).

Moreover when adjusted for vegetable consumption LEAD was found to be about 5.6 (3.8-8.2) times and 2.7 (1.8-4.1) times more prevalent (χ^2= 106.261, df 2; p<0.001) amongst participants of Gr I and Gr II respectively than amongst participants of Gr III.

Table-56: Distribution of LEAD among study participants by days of consumption of vegetable, study groups (I and II).

Study group	Vegetables consumed per week						OR (95% CI) adjusted for vegetables consumed per week
	less than 7 days a week			7 days a week			
	Total	LEAD		Total	LEAD		
		Yes	Prevalence (%)		Yes	Prevalence (%)	
Gr I	40	6	15.0	960	162	16.9	2.052 (1.560-2.699)
Gr II	72	5	6.9	927	84	9.1	1
Total	112	11	9.8	1887	246	13.0	χ^2= 28.617, df 2; p<0.001
Significance	Fisher's Exact value=0.196			χ^2= 25.396, df 1; p<0.001			

Amongst the 1999 study participants only about 5.6% (112) consumed vegetables less than 7 days a week. The prevalence of LEAD amongst those who consumed vegetables less than 7 days a week was found to be 9.8% whereas that amongst those who consumed vegetables 7 days a week was found to be 13.0 % And this observed difference in the prevalence between the vegetable consumption groups was not statistically significant (p= 0.323). In both the vegetable consumption groups the prevalence of LEAD was higher amongst participants of Gr I than amongst participants of Gr II, but only the difference in prevalence between the study groups amongst those who consumed vegetables 7days a week was found to be statistically significant (p<0.05).

Moreover when adjusted for vegetable consumption LEAD was found to be about 2.1 (1.6-2.7) times more prevalent (χ^2= 28.617, df 2; p<0.001) amongst participants of Gr I participants than amongst participants of Gr II.

Table-57: Distribution of LEAD among participants of by study groups and protein (fish, meat or egg) consumption.

Fish/ meat/ egg consumed per week	Study group	Total	LEAD		Significance
			Yes	Prevalence (%)	
<4 days a week (n=2251)	Gr I	756	130	17.2	χ^2= 77.399, df 2; p<0.001
	Gr II	791	73	9.2	
	Gr III	704	24	3.4	
≥4 days a week (n=748)	Gr I	244	38	15.6	χ^2= 24.061, df 2; p<0.001
	Gr II	208	16	7.7	
	Gr III	296	11	3.7	
OR (95% CI) adjusted for egg meat fish consumption	Gr I	5.538 (3.802-8.067)			χ^2= 106.342, df 2; p<0.001
	Gr II	2.672 (1.787-3.995)			
	Gr III	1			

Amongst the 2999 study participants only about 75% (2251) consumed fish, meat or egg for less than 4days a week and the remaining 25% (748) had higher protein consumption. Amongst those who consumed fish, meat or egg for less than 4days a week 33.6% (756) were members of Gr I while another 35.1% (791) and 31.3% (704) were members of Gr II and Gr III respectively. The prevalence of LEAD of among participants who consumed fish, meat or egg for less than 4days a week was about 10.1% (227). LEAD was found to be more prevalent in participants of Gr I (17.2%) than in participants of Gr II (9.2%) or participants in Gr III (3.4%) And the difference between the groups was found to be statistically significant ($p<0.05$).

Amongst those who consumed fish, meat or egg for 4 or more days a week 32.6% (244) were members of Gr I while another 27.8% (208) and 39.6% (296) were members of Gr II and Gr III respectively. Among them LEAD was found to be more prevalent among members of Gr I (15.6%) than among members of either Gr II (7.7%) or members of Gr III (3.7%). And the difference between the groups was found to be statistically significant ($p<0.05$).

When adjusted for fish meat egg consumption, LEAD was found to be about 5.5 (3.8-8.1) times and 2.7 (1.8-4.0) times more prevalent (χ^2= 106.342, df 2; $p<0.001$) amongst participants of Gr I and Gr II respectively than amongst participants of Gr III.

Table-58: Distribution of LEAD among participants by study groups (I and II) and protein (fish, meat or egg) consumption.

Egg/ meat/ fish consumed per week	Study group	Total	LEAD		Significance
			Yes	Prevalence (%)	
<4 days (n=1547)	Gr I	756	130	17.3	χ^2= 21.522, df 1; p<0.001
	Gr II	791	73	9.2	
≥4 days (n=452)	Gr I	244	38	15.6	χ^2= 6.630, df 1; p<0.01
	Gr II	208	16	7.7	
OR (95% CI) adjusted for fish meat egg consumption	Gr I	2.076 (1.578-2.730)			χ^2= 28.969, df 2; p<0.001
	Gr II	1			

Amongst the 1999 study participants only about 77.4% (1547) consumed fish meat or egg for less than 4 days a week. The prevalence of LEAD amongst those who consumed fish meat or egg for less than 4 days a week was found to be 13.1% whereas that amongst those who consumed fish meat or egg for 4 or more days a week was found to be 11.9 %. Amongst those who consumed fish meat or egg for less than 4 days a week LEAD was more prevalent in members of Gr I (17.3%) than among members of Gr II (9.2%), and this observed difference was found to be statistically significant ($p<0.05$).

LEAD was found to be prevalent amongst 11.5% of the participants who consumed fish meat or egg for 4 or more days a week. Higher proportion of participants had LEAD if they were members of Gr I (15.6) in contrast to those who were members of Gr II (7.7%). Again the difference in the prevalence of LEAD between the members of these study groups was found to be statistically significant ($p<0.05$).

Moreover when adjusted for fish meat egg consumption LEAD was found to be about 2.1 (1.6-2.7) times higher amongst participants of Gr I compared to that amongst participants of Gr II (χ^2= 28.969, df 2; $p<0.001$).

Table-59: Distribution of LEAD among participants by arsenic exposure groups and protein (fish, meat or egg) consumption.

Egg meat fish consumed per week	Excess Arsenic exposure	Total	LEAD		Significance
			Yes	Prevalence (%)	
<4 days (n=2251)	Yes	1547	203	13.1	χ^2= 50.340, df 1; p<0.001
	No	704	24	3.4	
≥4 days (n=748)	Yes	452	54	11.9	χ^2= 15.271, df 1; p<0.001
	No	296	11	3.7	
OR (95% CI) adjusted for fish meat egg consumption	Yes	4.047 (2.817- 5.814)			χ^2= 77.907, df 2; p<0.001
	No	1			

Out of the total 2999 study about 75% (2251) participants had excess arsenic exposure. The prevalence of LEAD amongst those who consumed fish meat or egg for less than 4 days a week was found to be 10.1% whereas that amongst those who consumed fish meat or egg for 4 or more days a week was found to be 8.7 %.

Amongst those who consumed fish meat or egg for less than 4 days a week LEAD was more prevalent those who had excess arsenic exposure (13.1%) than among those who did not have such exposure (3.4%), and this observed difference was found to be statistically significant ($p<0.05$). Again higher proportion of participants had LEAD if they had excess arsenic exposure (11.9) in contrast to those who had no such exposure (3.7%). Again the difference in the prevalence of LEAD between the members of these study groups was found to be statistically significant ($p<0.05$). Moreover when adjusted for fish meat egg consumption LEAD was found to be about 4.0 (2.8-5.8) times higher amongst participants having excess arsenic exposure compared to those having no such exposure (χ^2= 77.907, df 2; $p<0.001$).

Table-60: Distribution of LEAD among participants by study groups and smoking status (ever/never smoker).

Smoking status	Study group	Total	LEAD		Significance
			Yes	Prevalence (%)	
Ever smoker (n=613)	Gr I	203	32	15.8	χ^2= 14.659, df 2; p<0.001
	Gr II	202	23	11.4	
	Gr III	208	9	4.3	
Never smoker (n=2386)	Gr I	797	136	17.1	χ^2= 89.544, df 2; p<0.001
	Gr II	797	66	8.3	
	Gr III	792	26	3.3	
OR (95% CI) adjusted for smoking status	Gr I	5.572 (3.826-8.114)			χ^2= 106.328, df 3; p<0.001
	Gr II	2.699 (1.806-4.032)			
	Gr III	1			

Among the 613 ever smokers 27.9% (203) were members of Gr I, while the remaining 29.2% and 32.9% were members of Gr II and Gr III respectively. Among the ever smokers 15.8%, 11.4% and 4.3% in Gr I, Gr II and Gr III respectively were found to have LEAD and this observed difference in prevalence between the study groups was found to be statistically significant ($p<0.05$).

Among the 2386 participants who had never smoked 9.6% (228) were found to have LEAD. Members of Gr I (797) accounted for 33.4% of the never smokers, another 33.4% were members of Gr II and the remaining 33.2% were members of Gr III. Among never smokers LEAD was more prevalent amongst members of Gr I (17.1%) than amongst members of Gr II (8.3%) and Gr III (3.3%). And the observed difference between the study groups was found to be statistically significant ($p<0.05$).

Moreover when adjusted for smoking status, LEAD was found to be 5.6 (3.8-8.1) and 2.7 (1.8-4.0) times more prevalent amongst members of Gr I and Gr II respectively compared to that in Gr III (χ^2= 106.328, df 3; $p<0.001$)

Table-61: Distribution of LEAD among participants by study groups (Gr I and Gr II) and smoking status (ever/never smoker).

Smoking status	Study group	Total	LEAD		Significance
			Yes	Prevalence (%)	
Ever smoker (n=405)	Gr I	203	32	15.8	χ^2= 1.653, df 1; p=0 .199
	Gr II	202	23	11.4	
Never smoker (n=1594)	Gr I	797	136	17.1	χ^2= 27.778, df 1; p<0.001
	Gr II	797	66	8.3	
OR (95% CI) adjusted for smoking status	Gr I	2.065 (1.570-2.714)			χ^2= 28.408, df 2; p<0.001
	Gr II	1			

Among the 1999 study participants in Gr I and Gr II, 20.1% (405) were ever smokers and the other 79.1% (1594) were never smokers. LEAD was found amongst 13.6% (55) ever smokers and 12.7% (202) of never smokers. Amongst ever smokers almost equal numbers belonged to Gr I and Gr II. Amongst those who had ever smoked, 15.8% had LEAD if they were members of Gr I whereas 11.4 % had LEAD if they were members of Gr II. The observed difference in the prevalence of LEAD between the study groups amongst the ever smokers was not found to be statistically significant (p>0.05).

Among the never smokers LEAD was more prevalent amongst members of Gr I (17.1%) than amongst members of Gr II (8.3%). And the observed difference between the study groups was found to be statistically significant (p<0.05).

Moreover when adjusted for smoking status LEAD was found to be 2.1 (1.6-2.7) times more prevalent amongst members of Gr 1 compared to that among participants in Gr II (χ^2= 28.408, df 2; p<0.001).

Table-62: Distribution of LEAD among participants by excess arsenic exposure and smoking status (ever/never smoker).

Smoking status	Excess Arsenic exposure	Total	LEAD		
			Yes	Prevalence (%)	
Ever smoker (n=613)	Yes	405	55	13.6	χ^2= 12.584, df 1; p<0.001
	No	208	9	4.3	
Never smoker (n=2386)	Yes	1594	202	12.7	χ^2= 53.976, df 1 p<0.001
	No	792	26	9.6	
OR (95% CI) adjusted for smoking status	Yes	4.071 (2.835-5.846)			χ^2= 78.153, df 2; p<0.001
	No	1			

Among the 613 ever-smokers, 66.1% (405) had excess arsenic exposure while the remaining 33.9% did not have such exposure. LEAD was found to be more common amongst those having excess arsenic exposure (13.6%) than amongst those not having such exposure (4.3%). And this difference was found to be statistically significant ($p<0.05$).

Among the 2386 never-smokers 66.8% (1594) had excess arsenic exposure. LEAD was more prevalent among those having excess arsenic exposure (12.7%) than among those having no such exposure (9.6%). Again the difference between the exposure groups was found to be statistically significant ($p<0.05$).

Moreover when adjusted for smoking status LEAD was found to be 4.0 (2.8-5.8) times more prevalent amongst those having excess arsenic exposure compared to those having no such exposure (χ^2= 78.153, df 2; $p<0.001$).

Table-63: Odds ratio and their % CI of LEAD among the study groups adjusted for different food habit related variables and smoking status

Gender / Study group	Combined			Male			Female		
	Gr III	Gr II	Gr I	Gr III	Gr II	Gr I	Gr III	Gr II	Gr I
Crude	1	2.697 (1.80-4.03)	5.567 (3.82-8.11)	1	2.328 (1.219-4.445)	5.464 (3.026-9.864)	1	2.946 (1.764-4.920)	5.651 (3.472-9.199)
Food habit related variables									
Adjusted for staple food type	1	2.866 (1.903-4.318)	5.926 (4.031-8.711)	1	2.444 (1.264-4.726)	5.718 (3.130-10.446)	1	3.178 (1.881-5.367)	6.141 (3.717-10.144)
Adjusted for vegetable intake (days per wk)	1	2.756 (1.842-4.125)	5.628 (3.863-8.200)	1	2.424 (1.265-4.643)	5.573 (3.078-10.090)	1	2.932 (1.750-4.912)	5.640 (3.4649.181)
Adjusted for protein intake (days per wk)	1	2.683 (1.795-4.010)	5.551 (3.811-8.084)	1	2.056 (1.063-3.976)	5.061 (2.781-9.212)	1	2.806 (1.672-4.708)	5.417 (3.317-8.847)
Adjusted for milk or milk product intake (days per wk)	1	2.681 (1.795-4.006)	5.626 (3.861-8.197)	1	2.334 (1.220-4.463)	5.887 (3.245-10.681)	1	2.769 (1.649-4.650)	5.534 (3.392-9.027)
Adjusted for cooking oil	1	2.697 (1.805-4.028)	5.569 (3.825-8.110)	1	2.365 (1.237-4.519)	5.411 (2.996-9.774)	1	2.971 (1.779-4.963)	5.681 (3.489-9.250)
Adjusted for all food habits	1	2.973 (1.968-4.490)	6.254 (4.233-9.238)	1	2.629 (1.350-5.120)	6.375 (3.445-11.797)	1	3.154 (1.857-5.357)	6.100 (3.675-10.124)
Adjusted for all food habits age & gender	1	3.114 (2.054-4.721)	6.766 (4.557-10.047)						
Tobacco consumption related variables									
Adjusted for smoking status				1	2.346 (1.227-4.486)	5.573 (3.082-10.076)			
Adjusted for smoking status and years of smoking				1	2.470 (1.283-4.756)	5.922 (3.241-10.823)			
Adjusted for habit of tobacco chewing	1	2.682 (1.794-4.010)	5.533 (3.796-8.067)	1	2.343 (1.226-4.478)	5.513 (3.048-9.970)	1	2.889 (1.727-4.832)	5.545 (3.402-9.041)
Adjusted for tobacco consumption & duration of tobacco consumption	1	2.749 (1.828-4.136)	5.694 (3.871-8.375)	1	2.274 (1.178-4.389)	5.484 (2.993-10.049)	1	3.080 (1.825-5.200)	6.030 (3.643-9.981)
Adjusted for age gender tobacco consumption, & duration of tobacco consumption.	1	2.706 (1.795-4.081)	5.722 (3.879-8.441)						

A series of logistic regression analyses were undertaken using presence or absence of LEAD as the binary dependent variable to determine the OR (95%CI) LEAD for different study groups categories adjusted for different food habit and tobacco consumption related variables. The results are presented in table 47.

Adjustments were done for all food habits related variables (staple food type, numbers of days of vegetable intake per week, number of days of fish/meat/egg intake per week, numbers of days of milk or milk products intake per week and cooking oil) individually, thereafter adjustment was done for all these variables together, and finally all food habit related variables, age and gender together. Similar adjustments were undertaken for the tobacco consumption related variables (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco).

It was found that LEAD was 5.6 and 2.7 times more prevalent (χ^2=105.818, df 2; p<0.001) among participants of Gr I (having arsenicosis in addition to excess arsenic exposure) and Gr II (those having excess arsenic exposure but no arsenicosis) respectively compared to those not having excess arsenic exposure (Gr III). Amongst males LEAD was found to be 5.5 and 2.3 more prevalent (χ^2=43.787, df 2; p<0.001) among participants of Gr I and Gr II respectively compared to that among those belonging to Gr III. Among females LEAD was found to be 5.6 and 2.9 more prevalent (χ^2=62.992, df 2; p<0.001) among participants of Gr I and Gr II respectively compared to that among those belonging to Gr III. The odds of LEAD for the different study groups were found to be a bit higher among females than among males.

When adjusted for all food habit related variables together LEAD was found to be 6.2 and about 3.0 more prevalent (χ^2=111.970, df 8; p<0.001) among participants of Gr I and Gr II respectively compared to those belonging to Gr III. Amongst males LEAD was found to be 6.4 and 2.6 more prevalent (χ^2=52.808, df 7; p<0.001) among participants of Gr I and Gr II respectively compared to that among those belonging to Gr III. While among females LEAD was found to be 6.1 and 3.1 times more prevalent (χ^2=68.429, df 7; p<0.001) among participants of Gr I and Gr II respectively compared to that among those belonging to Gr III. The odds of LEAD for the different study groups were found to be a bit higher among males than among females. Finally again when adjusted for all

food habit related variables, age and gender together LEAD was found to be 6.8 and about 3.1 more prevalent (χ^2=177.437, df 9; p<0.001) among participants of Gr I and Gr II respectively compared to those belonging to Gr III.

Tobacco consumption in terms of smoking was only prevalent amongst the male participants of the study. Whether adjusted for smoking status or smoking status and years of smoking the adjusted ORs was found to be higher than the crude ORs

As tobacco consumption in terms of smoking and chewing was prevalent among the study participants adjustment for all tobacco consumption related variables (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco) showed LEAD was found to be 5.7 and about 2.7 more prevalent (χ^2= 107.685, df 7; p<0.001) among participants of Gr I and Gr II respectively compared to those belonging to Gr III. Amongst males LEAD was found to be 5.5 and 2.3 more prevalent (χ^2= 55.705, df 7; p<0.001).among participants of Gr I and Gr II respectively compared to that among those belonging to Gr III. Among females LEAD was found to be 6.0 and 3.1 more prevalent (χ^2= 66.237, df 4; p<0.001) among participants of Gr I and Gr II respectively compared to that among those belonging to Gr III. The odds of LEAD for the different study groups were found to be a bit higher among females than among males.

Finally when adjusted for all tobacco consumption related variables, age and gender together, LEAD was found to be about 5.7 and 2.7 times ore prevalent (χ^2= 181.535, df 9; p<0.001) among participants of Gr I and Gr II respectively compared to those belonging to Gr III.

4.4 MORBIDITY RELATED VARIABLES AND LEAD AMONG THE STUDY GROUPS

Table-64: Distribution of LEAD among participants by study groups, overweight/obesity and gender.

Overweight or Obese	Study group	Gender of participant					
		Male		Female		Combined	
		LEAD		LEAD		LEAD	
		Yes	No	Yes	No	Yes	No
Yes	Gr I	2 (11.1)	16	8 (16.3)	41	10 (14.9)	57
	Gr II	0 (0)	22	6 (9.5)	57	6 (7.1)	79
	Gr III	1 (4.0)	24	3 (4.3)	67	4 (4.2)	91
No	Gr I	69 (16.6)	346	89 (17.2)	429	158 (16.9)	775
	Gr II	31 (8.2)	349	52 (9.7)	482	83 (9.1)	831
	Gr III	13 (3.4)	366	18 (3.4)	508	31 (3.4)	874
OR (95% CI) adjusted for overweight or obesity	Gr I	5.414 (2.998- 9.777)		5.651 (3.471- 9.202)		5.549 (3.810-8.082)	
	Gr II	2.320 (1.215- 4.432)		2.946 (1.764- 4.921)		2.694 (1.803- 4.024)	
	Gr III	1		1		1	
OR (95% CI) adjusted for gender and overweight or obesity	Gr I	5.567 (3.822- 8.109)					
	Gr II	2.693 (1.803- 4.023)					
	Gr III	1					

Among the 2999 study participants 1239 (41.3%) were male and the rest 1760 (58.7%) were female. Of the 2999 study participants 8.2% (247) were either overweight or obese and the other 91.8% (2752) were neither overweight nor obese. Amongst those who were overweight or obese 8.1% (20) had LEAD, on the other hand LEAD was found to be prevalent amongst 9.9% (272) of those, who were neither overweight nor obese. Amongst those who were overweight or obese, members of Gr I, Gr II and Gr III accounted for 27.1% (67), 34.4% (85) and 38.5% (95) of the participants respectively. Again amongst those who were neither overweight nor obese members of Gr I, Gr II and Gr III accounted for 33.9% (933), 33.2% (914) and 32.9% (905) of the participants respectively. Amongst those who were overweight or obese prevalence of LEAD found to be higher in Gr I (14.9%) and Gr II (7.1%) participants than in Gr III (4.2%) participants, and the observed difference was statistically significant (χ^2=6.249, df 2;

p=0.044). Again amongst those who were neither overweight nor obese prevalence of LEAD found to be higher in Gr I (16.9%) and Gr II (9.1%) participants than in Gr III (3.4%) participants, and the observed difference was statistically significant (χ^2= 95.119, df 2; p<0.001). When adjusted for overweight or obese LEAD was found to be about 5.5 (3.8-8.1) and 2.7 (1.8-4.0) times higher (χ^2= 106.084, df 3; p<0.001) amongst members of Gr I and Gr II respectively in contrast to participants of Gr III.

Among the 1239 male participants 5.2% (65) were either overweight or obese and the other 95.8% (1174) were neither overweight nor obese. Amongst those who were overweight or obese 4.6% (4) had LEAD, on the other hand LEAD was found to be prevalent amongst 9.6% (113) of those who were neither overweight nor obese. Amongst those who were overweight or obese, 11.1% (2) and 4.0% (1) of the participants of Gr I, and Gr III respectively had LEAD and the observed difference in LEAD was not found to be statistically significant (Fisher's Exact value = 2.415, p= 0 .271). Again amongst those who were neither overweight nor obese prevalence of LEAD found to be higher in Gr I (16.6%) and Gr II (8.2%) participants than in Gr III (3.4%) participants, and the observed difference was statistically significant (χ^2= 41.048, df 2; p<0.001). When adjusted for overweight or obese LEAD was found to be about 5.4 (3.0-9.8) and 2.3 (1.2-4.4) times higher (χ^2= 45.38, df 3; p<0.001) amongst male members of Gr I and Gr II respectively in contrast to male participants of Gr III.

Among the 1760 female participants 10.3% (182) were either overweight or obese and the remaining 89.7% (1578) were neither overweight nor obese. Amongst those who were overweight or obese 9.3% (17) had LEAD, on the other hand LEAD was found to be prevalent amongst 10.1% (159) of those who were neither overweight nor obese. Amongst those who were overweight or obese, 16.3% (8), 9.5% (6) and 4.3% (3) of the participants of Gr I, Gr II and Gr III respectively had LEAD and the observed difference in LEAD was not found to be statistically significant (χ^2=4.939, df 2; p= 0 .085). Again amongst those who were neither overweight nor obese prevalence of LEAD found to be higher in Gr I (17.2%) and Gr II (9.7%) participants than in Gr III (3.4%) participants, and the observed difference was statistically significant (χ^2= 54.634, df 2; p<0.001). When adjusted for overweight or obese LEAD was found to be about 5.6 (3.5-9.2) and

2.9 (1.8-4.9) times higher (χ^2= 62.992, df 3; p<0.001) amongst female members of Gr I and Gr II respectively in contrast to female participants of Gr III.

Finally when adjusted for overweight or obesity and gender, LEAD was found to be about 5.6 (3.8-8.1) and 2.7 (1.8-4.0) times higher (χ^2=106.897, df 4; p<0.001) amongst members of Gr I and Gr II respectively in comparison to that among members of Gr III.

Table-65: Distribution of respondents by age, gender, overweight/obesity, study groups and LEAD.

Age group (years)	Overweight or Obese	Study group	Gender								
			Male			Female			Combined		
			LEAD		Total	LEAD		Total	LEAD		Total
			Yes	No		Yes	No		Yes	No	
30-39	Yes	Gr I	1	6	7	3	21	24	4	27	31
		Gr II	0	12	12	2	24	26	2	36	38
		Gr III	0	10	10	1	36	37	1	46	47
	No	Gr I	22	167	189	38	236	274	60	403	463
		Gr II	6	198	204	22	285	307	28	483	511
		Gr III	0	154	154	4	277	281	4	431	435
40-49	Yes	Gr I	0	6	6	4	15	19	4	21	25
		Gr II	0	6	6	3	25	28	3	31	34
		Gr III	0	11	11	1	22	23	1	33	34
	No	Gr I	29	118	147	35	149	184	64	267	331
		Gr II	10	87	97	19	148	167	29	235	264
		Gr III	4	133	137	5	168	173	9	301	310
50-60	Yes	Gr I	1	4	5	1	5	6	2	9	11
		Gr II	0	4	4	1	8	9	1	12	13
		Gr III	1	3	4	1	9	10	2	12	14
	No	Gr I	18	61	79	16	44	60	34	105	139
		Gr II	15	64	79	11	49	60	26	113	139
		Gr III	9	79	88	9	63	72	18	142	160
Total			116	1123	1239	176	1584	1760	292	2707	2999
OR (95% CI) adjusted for age and overweight/obesity		Gr I	6.038 (3.318-10.986)			5.877 (3.596-9.604)			5.880 (4.024-8.593)		
		Gr II	2.625 (1.364-5.052)			3.074 (1.835-5.149)			2.884 (1.925-4.322)		
		Gr III	1			1			1		
OR (95% CI) adjusted for age, gender, and overweight/obesity,		Gr I	5.923 (4.052-8.658)								
		Gr II	2.882 (1.923-4.319)								
		Gr III	1								

Among the 2999 study participants the proportion of respondents in the age stratum (years) 30-39, 40-49 and 50-60 were 50.8% (1525), 33.3% (988), and 15.9% (476) respectively. Among all study participants 8.2% (247) were either overweight or obese and the other 91.8% (2752) were neither overweight nor obese.

When adjusted for age (grouped data) and obesity/overweight LEAD was found to be about 5.9 and 2.9 times more prevalent (χ^2=159.895, df 5; p<0.001) among participants of Gr I and Gr II respectively than amongst participants of Gr III. Among the female participants of the study LEAD was found to be about 5.9 and 3.1 times more common (χ^2=86.627, df 5; p<0.001) among participants of Gr I and Gr II respectively than amongst participants of Gr III. On the other hand among males LEAD was found to be about 6.0 and 2.6 times more prevalent (χ^2=79.584, df 5; p<0.001) among participants of Gr I and Gr II respectively than amongst participants of Gr III.

Finally when adjusted for the differences in age, gender and obesity/overweight between the study groups it was found that LEAD was about 5.9 and 2.8 times more prevalent (χ^2= 163.007, df 6; p<0.001) among participants of Gr I and Gr II respectively than among participants of Gr III.

Table-66: Distribution of respondents by age, gender, overweight/obesity, study groups (I & II) and LEAD.

Age group (years)	Overweight or Obese	Study group	Gender								
			Male			Female			Combined		
			LEAD		Total	LEAD		Total	LEAD		Total
			Yes	No		Yes	No		Yes	No	
30-39	Yes	Gr I	1	6	7	3	21	24	4	27	31
		Gr II	0	12	12	2	24	26	2	36	38
	No	Gr I	22	167	189	38	236	274	60	403	463
		Gr II	6	198	204	22	285	307	28	483	511
40-49	Yes	Gr I	0	6	6	4	15	19	4	21	25
		Gr II	0	6	6	3	25	28	3	31	34
	No	Gr I	29	118	147	35	149	184	64	267	331
		Gr II	10	87	97	19	148	167	29	235	264
50-60	Yes	Gr I	1	4	5	1	5	6	2	9	11
		Gr II	0	4	4	1	8	9	1	12	13
	No	Gr I	18	61	79	16	44	60	34	105	139
		Gr II	15	64	79	11	49	60	26	113	139
Total			102	733	835	155	1009	1164	257	1742	1999
OR (95% CI) adjusted for age and overweight/obesity		Gr I	2.270 (1.441-3.576)			1.902 (1.340-2.700)			2.022 (1.534-2.666)		
		Gr II	1			1			1		
OR (95% CI) adjusted for age, gender, and overweight/obesity,		Gr I	2.38 (1.545-2.688)								
		Gr II	1								

The prevalence of LEAD amongst male respondents who were 30-39 years of age and were not overweight or obese was 5.3% and 7.1% amongst those who were not overweight or obese had LEAD. The difference in prevalence of LEAD between Gr I and Gr II among those who were overweight or obese was not statistically significant (Fisher's exact value = 0.368). and the difference in prevalence of LEAD between Gr I and Gr II among those who were not overweight or obese was found to be statistically significant (χ^2= 11.220, df 1; p=0.001).

For the same age stratum, amongst females the prevalence of LEAD amongst those who were overweight or obese was 10.0% and among those who were non over weight or non obese the prevalence was 7.2%. The difference in prevalence of LEAD between Gr I and Gr II among those who were overweight or obese was not statistically significant (Fisher's exact value = 0.661). However the difference in prevalence of LEAD between

Gr I and Gr II among those who were non weight or non obese was found to be statistically significant (χ^2 = 7.023, df 1; p=0.008).

No male respondents in 40-49 years of age who were overweight or obese were found to have LEAD. But amongst those who were not overweight or obese, the prevalence was found to be 16.0%. And the difference in prevalence of LEAD between Gr I and Gr II among those who were non overweight or non obese was found to be just statistically significant (χ^2 = 3.860, df 1; p=0.049).

For the same age stratum, amongst females the prevalence of LEAD amongst those who were overweight or obese was 14.9% and among those who were non over weight or non obese the prevalence was 15.4%. The difference in prevalence of LEAD between Gr I and Gr II among those who were overweight or obese was not statistically significant (Fisher's exact value = 0.417). However the difference in prevalence of LEAD between Gr I and Gr II among those who were non weight or non obese was found to be statistically significant (χ^2 = 3.930, df 1; p=0.047).

The prevalence of LEAD amongst male respondents who were 50-60 years of age and were overweight or obese was 11.1% and amongst those whose were not overweight or obese the prevalence was 20.8%. Moreover the difference in prevalence of LEAD between Gr I and Gr II among those who were overweight or obese was not statistically significant (Fisher's exact value=1.0). However the difference in prevalence of LEAD between Gr I and Gr II among those who were non overweight or non-obese was found not to be statistically significant (χ^2 =0.349, df 1; p=0.557).

For the same age stratum amongst females the prevalence of LEAD amongst those who were overweight or obese was 13.3% and among those who were non overweight or non obese the prevalence was 22.5%. The difference in prevalence of LEAD between Gr I and Gr II among those who were overweight or obese was not statistically significant (Fisher's exact value = 1.0). However the difference in prevalence of LEAD between Gr I and Gr II among those who were non overweight or non obese was also found not to be statistically significant (χ^2 = 1.195, df 1; p= 0.274).

Finally when stratified by and adjusted for age & obesity/overweight, LEAD was found to be 2.0 times higher (χ^2 =60.564, df 4; p=0.001) amongst Gr I participants compared to Gr II participants. When adjusted for age & obesity/overweight, LEAD was 2.3 and 1.9

times more prevalent amongst Gr I participants than amongst Gr II participants in amongst males (χ^2 =38.880, df 4; p<0.001) and females (χ^2 =26.955, df 4; p=0.001) respectively.

Finally when adjusted for age, gender, & obesity/overweight LEAD was still found to be about 2.0 times more prevalent (χ^2= 62.948,df 5; p=0.001) amongst Gr I participants than amongst Gr II participants.

Table-67: Distribution of respondents by age, gender, overweight/obesity, excess arsenic exposure and LEAD

Age group (years)	Overweight or Obese	Excess Arsenic exposure	Gender								
			Male			Female			Combined		
			LEAD		Total	LEAD		Total	LEAD		Total
			Yes	No		Yes	No		Yes	No	
30-39	Yes	Yes	1	18	19	5	45	50	6	63	69
		No	0	10	10	1	36	37	1	46	47
	No	Yes	28	365	393	60	521	581	7	52	59
		No	0	154	154	4	277	281	1	33	34
40-49	Yes	Yes	0	12	12	7	40	47	3	21	24
		No	0	11	11	1	22	23	2	12	14
	No	Yes	39	205	244	54	297	351	88	886	974
		No	4	133	137	5	168	173	4	431	435
50-60	Yes	Yes	1	8	9	2	13	15	93	502	595
		No	1	3	4	1	9	10	9	301	310
	No	Yes	33	125	158	27	93	120	60	218	278
		No	9	79	88	9	63	72	18	142	160
Total			116	1123	1239	176	1584	1760	292	2707	2999
OR (95% CI) adjusted for age and overweight/obesity		Yes	4.338 (2.433-7.734)			4.377 (2.735-7.004)			4.323 (3.003-6.225)		
		No	1			1			1		
OR (95% CI) adjusted for age, gender, and overweight/obesity,		Yes	4.335 (3.010-6.243)								
		No	1								

The prevalence of LEAD amongst male respondents who were 30-39 years of age and were overweight or obese was 3.4% and amongst those whose were not overweight or obese the prevalence was 5.1%. Moreover the difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure, among those who were overweight or obese was not statistically significant (Fisher's exact value=1.0).

However the difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure, among those who were not overweight or obese was found to be statistically significant (χ^2 = 11.564, df 1; p=0.001).

For the same age stratum amongst females, the prevalence of LEAD amongst those who were overweight or obese was 6.9% and among those who were non overweight or non obese the prevalence was 7.4%. The difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure, among those who were overweight or obese was not statistically significant (Fisher's exact value = 0.234). However the difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure, among those who were not overweight or obese was found to be statistically significant (χ^2 = 21.844, df 1; p<0.001).

No overweight or obese male respondents, 40-49 years of age were found to have LEAD. But amongst those whose were not overweight or obese the prevalence was found to be 11.3%. And the difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure among those who were not overweight or obese was found to be statistically significant (χ^2 = 14.955, df 1; p<0.001).

For the same age stratum amongst females the prevalence of LEAD amongst those who were overweight or obese was 11.4% and among those who were non over weight or non obese the prevalence was 11.3%. The difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure, among those who were overweight or obese was not statistically significant (χ^2_c =0.815, df 1; p=0.193).However the difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure, among those who were not overweight or obese was found to be statistically significant (χ^2 = 18.106, df 1; p<0.001).

The prevalence of LEAD amongst male respondents who were 50-60 years of age and were overweight or obese was 15.4% and amongst those whose were not overweight or obese the prevalence was 17.1%. Moreover the difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure, among those who were overweight or obese was not statistically significant (Fisher's exact value=1.0). However the difference in prevalence of LEAD between those having

excess arsenic exposure and those not having such exposure among those who were not overweight or obese was found to be statistically significant (χ^2 = 4.535, df 1; p=0.033).

For the same age stratum amongst females the prevalence of LEAD amongst those who were overweight or obese was 12.0% and among those who were non overweight or non obese the prevalence was 18.8%. The difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure, among those who were overweight or obese was not statistically significant (Fisher's exact value = 1.0). However the difference in prevalence of LEAD between those having excess arsenic exposure and those not having such exposure, among those who were not overweight or obese was not found to be statistically significant (χ^2 = 0.063, df 1; p=0.82).

Finally when stratified by and adjusted for age & obesity/overweight, LEAD was found to be 4.3 times higher (χ^2=133.514, df 4; p<0.001) amongst participants those having excess arsenic exposure compared to those not having such exposure participants. Amongst males when adjusted for age & obesity/overweight, LEAD was 4.3 times more prevalent (χ^2 =65.941, df 4;p<0.001) amongst participants those having excess arsenic exposure than amongst those participants not having such exposure; while in females after such adjustment LEAD was found to be about 4.4 times more prevalent (χ^2= 73.187, df 4; p<0.001) amongst participants those having excess arsenic exposure than amongst those not having such exposure participants and females.

Finally when adjusted for age, gender, & obesity/overweight LEAD was still found to be about 4.3 times more prevalent (χ^2 =136.075, df 5; p<0.001) amongst those having excess arsenic exposure participants than amongst participants not having such exposure.

Table-68: Distribution of respondents by diabetic status, gender, study groups and LEAD

Diabetes	Study group	Gender								
		Male			Female			Combined		
		Total	LEAD		Total	LEAD		Total	LEAD	
			Yes	Prev* (%)		Yes	Prev* (%)		Yes	Prev* (%)
Yes	Gr I	27	5	18.5	41	11	26.8	68	16	23.5
	Gr II	19	2	10.5	40	4	10.0	59	6	10.2
	Gr III	11	2	18.2	20	1	5.0	31	3	9.7
No	Gr I	406	66	16.3	526	86	16.3	932	152	16.3
	Gr II	383	29	7.6	557	54	9.7	940	83	8.8
	Gr III	393	12	3.1	576	20	3.5	969	32	3.3
Total		1239	116	9.4	1760	176	10.0	2999	292	9.7
OR (95% CI) adjusted for diabetic status	Gr I	5.471 (3.755-7.972)			5.369 (2.971-9.702)			5.555 (3.410-9.050)		
	Gr II	2.659 (1.779-3.974)			2.303 (1.205-4.400)			2.901 (1.736-4.847)		
	Gr III	1			1			1		
OR (95% CI) adjusted for diabetic status & gender	Gr I	5.491 (3.768-8.001)								
	Gr II	2.659 (1.779-3.974)								
	Gr III	1								

*Prevalence

Among the 2999 study participants 158 (5.3%) were diabetic, of them only 36.1% (57) were male. Among those who were diabetic 15.8% (25) had LEAD, whereas 9.4% (267) of those were no- diabetic had LEAD. Among the non diabetic participants LEAD was more prevalent among those of Gr I (16.3%) and Gr II (8.3%) than among those of Gr III (χ^2 =94.919, df 2; p<0.001). On the other hand among the diabetics, LEAD was more prevalent among those of Gr I (23.5%) and Gr II (10.2%) than among those of Gr III but the difference between the study groups was not statistically significant (χ^2 = 5.327, df 2; p=0.07). LEAD was found to be about 5.5 (3.7-8.0) and 2.6 (1.8-4.0) times more prevalent (χ^2 =109.133, df 3; p<0.001) among participants of Gr I and Gr II respectively than among participants of Gr III.

Among the 1760 female participants, 5.7% (101) were diabetic and 9.4% (116) had LEAD. LEAD was more prevalent among those who were diabetic (15.8%), than among those who were non diabetic (9.6%). Among those who were diabetic, LEAD was more prevalent among those of Gr I (26.8%) and Gr II (10.0%) than among those of Gr III and the difference between the study groups was not statistically significant (χ^2 =6.500,

df 2; p=0.039). Among the non diabetic participants LEAD was more prevalent (χ^2 =52.322, df 2; p<0.001) among those of Gr I (16.3%) and Gr II (9.7%) than among those of Gr III. Adjusted for diabetic status LEAD was found to be about 5.5 (3.4-9.0) and 2.9 (1.7-4.8) times more common (χ^2 =64.890, df3; p<0.001) among females participants of Gr I and Gr II respectively than among those of Gr III.

Among the 1239 male participants, 4.6% (57) were diabetic and 10.0% (176) had LEAD. LEAD was more prevalent among those who were diabetic (15.8%), than among those who were non diabetic (9.1%). Among those who were diabetic LEAD was more prevalent among those of Gr I (18.5%) and Gr II (10.5%) than among those of Gr III and the difference between the study groups was not statistically significant (Fisher's Exact value p=0.800). Among the non diabetic participants LEAD was more prevalent among those of Gr I (16.3%) and Gr II (7.6%) than among those of Gr III (χ^2 =43.789, df 2; p<0.001). Among males when adjusted for diabetic status, LEAD was found to be about 5.4 (3.0-9.7) and 2.3 (1.2-4.4) times more common (χ^2 =45.101, df 3; p<0.001) among participants of Gr I and Gr II respectively than among those of Gr III.

Finally when adjusted for gender and diabetic status LEAD was found to be about 5.4 (3.8-8.0) and 2.7 (1.8-4.0) times higher (χ^2 =109.779, df 4; p<0.001) among participant of Gr I and Gr II respectively than among participants of Gr III.

Table-69: Distribution of respondents by diabetic status, gender, study groups (I & II) and LEAD

Diabetes	Study group	Gender								
		Male			Female			Combined		
		Total	LEAD		Total	LEAD		Total	LEAD	
			Yes	Prev* (%)		Yes	Prev* (%)		Yes	Prev* (%)
Yes	Gr I	27	5	18.5	41	11	26.8	68	16	23.5
	Gr II	19	2	10.5	40	4	10.0	59	6	10.2
No	Gr I	406	66	16.3	526	86	16.3	932	152	16.3
	Gr II	383	29	7.6	557	54	9.7	940	83	8.8
Total		835	102	12.2	1164	155	13.2	1999	257	12.9
OR (95% CI) adjusted for diabetic status	Gr I	2.340 (1.497-3.656)			1.915 (1.352-2.713)			2.059 (1.566-2.707)		
	Gr II	1			1			1		
OR (95% CI) adjusted for diabetic status & gender	Gr I	2.067 (1.572-2.718)								
	Gr II	1								

*Prevalence

Among the 1999 participants of study groups I and II 6.3% (127) were diabetic, of them only 36.2% (46) were male. Among those who were diabetic 8.6% (22) had LEAD whereas 6% (105) of the non diabetic had LEAD. Among the non diabetic participants LEAD was more prevalent among those of Gr I (16.3%) than among those of Gr II (8.8%) and the difference was just statistically significant (χ^2 =3.937, df 1; p=0.047). On the other hand among those who were diabetic, LEAD was more prevalent among those of Gr I (23.5%) than among those of Gr II (10.2%), and the difference between the study groups was also statistically significant (χ^2 = 23.848, df 1; p<0.001). When adjusted for diabetic status LEAD was found to be about 2.1 (1.6-2.7) times more common (χ^2=30.141, df 2; p<0.001) among members of Gr I than among members of Gr II.

Among the 1760 female participants about 2.6% (46) were diabetic and 10.0% (176) had LEAD. LEAD was more prevalent among those who were diabetic (15.2%), than among those who were non diabetic (12.0%). Among those who were diabetic though LEAD was more prevalent among those of Gr I (13.6%) than among those of Gr II (10.0%), and

the difference between the study groups was not statistically significant (χ^2 =3.800, df 12; p=0.051). Among the non diabetic participants LEAD was more prevalent among those of Gr I (16.3%) than among those of Gr II (9.7%) and the difference was statistically significant (χ^2 =10.645, df 1; p=0.001). When adjusted for diabetic status LEAD was found to be about 1.9 (1.3-2.7) times more prevalent (χ^2=15.650, df 2; p<0.001) among members of Gr I than among members of Gr II.

Among the 835 male participants about 7.0% (81) were diabetic and 13.3% (102) had LEAD. LEAD was more prevalent among those who were diabetic (18.5%), than among those who were non diabetic (12.9%). Among those who were diabetic though LEAD was more prevalent among those of Gr I (18.5%) than among those of Gr II (10.5%), and the difference between the study groups was not statistically significant (Fisher's Exact value p=0.744). Among the non diabetic participants LEAD was more prevalent among those of Gr I (16.3%) than among those of Gr II (7.6%) and the difference was statistically significant (χ^2 =15.321, df 2; p<0.001).

When adjusted for diabetic status and gender LEAD was found to be about 2.1 (1.6-2.7) times more prevalent (χ^2=30.877, df 3; p<0.001) among members of Gr I than among members of Gr II.

Table-70: Distribution of respondents by diabetic mellitus (DM), gender, excess arsenic exposure and LEAD

Diabetes	Excess arsenic exposure	Gender								
		Male			Female			Combined		
		Total	LEAD		Total	LEAD			LEAD	
			Yes	Prev* (%)		Yes	Prev* (%)	Total	Yes	Prev* (%)
Yes	Yes	46	7	15.2	81	15	18.5	127	22	17.3
	No	11	2	18.2	20	1	5.0	31	3	9.7
No	Yes	789	95	12.0	1083	140	12.0	1872	235	12.6
	No	393	12	3.1	576	20	3.5	969	32	3.3
Total		1239	116	9.4	1760	176	10.0	2999	292	9.7
OR (95% CI) adjusted for DM	Yes	3.816 (2.152-6.765)			4.137 (2.591-6.605)			4.001 (2.785-5.749)		
	No	1			1			1		
OR (95% CI) adjusted for DM & gender	Yes	4.007 (2.789-5.757)								
	No	1								

* Prevalence

Among the 2999 study participants 158 (5.3%) were diabetic, of them only 36.1% (57) were male. Among those who were diabetic, 15.8% (25) had LEAD whereas 9.4% (267) of those where not diabetic had LEAD. Among the non diabetic participants LEAD was more prevalent (12.6%) among those with excess arsenic exposure than among with no such exposure (3.3%) and the difference was statistically significant (χ^2 =64.175, df 1; p<0.001). On the other hand among those who were diabetic, LEAD was more prevalent (17.3%) among those with excess arsenic exposure than among with no such exposure (9.7%), but the difference between the study groups was not statistically significant (χ^2 = 1.094, df 1; p=0.296). When adjusted for diabetic status LEAD was found to be about 4.0 (2.9-5.7) times more common (χ^2=81.271, df 2; p<0.001) among those having excess arsenic exposure than those having no such exposure.

Among the 1760 female participants about 5.7% (101) were diabetic and 10.0% (176) had LEAD. LEAD was more prevalent among those who were diabetic (15.8%), than among the non-diabetic (9.6%). Among those who were diabetic, though LEAD was more prevalent (18.5%) among those with excess arsenic exposure than among with no such exposure (5.0%), and the difference between the study groups was not statistically

significant (χ^2=2.199, df 1; p=0.138). Among the non diabetic participants LEAD was more prevalent (12.9%) among those with excess arsenic exposure than among with no such exposure (3.5%), and the difference was statistically significant (χ^2 =38.573, df 1; p<0.001). When adjusted for diabetic status LEAD was found to be about 4.1 (2.3-6.6) times more common (χ^2=51.108, df 2; p<0.001) among those having excess arsenic exposure than those having no such exposure.

Among the 1239 male participants about 4.6% (57) were diabetic and 9.4% (116) had LEAD. LEAD was more prevalent among those who were diabetic (15.1%), than among those who were non diabetic (9.1%). Among those who were diabetic though LEAD was less prevalent (15.2%) among those with excess arsenic exposure than among with no such exposure (18.2%), and the difference between the study groups was not statistically significant (χ^2=2.199, df 1; p=0.138). Among the non diabetic participants LEAD was more prevalent (12.0%) among those with excess arsenic exposure than among with no such exposure (3.1%), and the difference was statistically significant (χ^2 =25.736, df 1; p<0.001). When adjusted for diabetic status LEAD was found to be about 3.8 (2.1-6.8) times more common (χ^2=30.309, df 2; p<0.001) among those having excess arsenic exposure than those having no such exposure.

When adjusted for diabetic status and gender LEAD was found to be about 4.0 (2.8-5.7) times more prevalent (χ^2=81.681, df 3; p<0.001) among members having excess arsenic exposure than among members having no such exposure.

Table-71: Distribution of respondents by hypertension status, gender, study groups and LEAD

HTN	Study group	Gender								
		Male			Female			Combined		
		Total	LEAD		Total	LEAD		Total	LEAD	
			Yes	Prev* (%)		Yes	Prev* (%)		Yes	Prev* (%)
Yes	Gr I	28	2	7.1	38	7	18.4	66	9	13.6
	Gr II	32	4	12.5	30	2	6.7	62	6	9.7
	Gr III	13	2	15.4	23	2	8.7	36	4	11.1
No	Gr I	405	69	17.0	529	90	17.0	934	159	17.0
	Gr II	370	27	7.3	567	56	9.9	937	83	8.9
	Gr III	391	12	3.1	573	19	3.3	964	31	3.2
Total		1239	116	9.4	1760	176	10.0	2999	292	9.7
OR (95% CI) adjusted for HTN status	Gr I	5.450 (3.017-9.844)			5.636 (3.461-9.178)			5.555 (3.813-8.091)		
	Gr II	2.319 (1.213-4.433)			2.943 (1.762-4.915)			2.691 (1.801-4.021)		
	Gr III	1			1			1		
OR (95% CI) adjusted for HTN status & gender	Gr I	5.574 (3.826-8.120)								
	Gr II	2.691 (1.801-4.021)								
	Gr III	1								

* Prevalence

Among the 2999 study participants 164 (5.5%) were hypertensive, of them only 44.5% (73) were male. The prevalence of HTN was 6.6%, 6.2% and 3.6% among participants of Gr I, Gr II and Gr III respectively. And the groups were found to be statistically different (χ^2 =10.282, df 2; p=0.006) in terms of HTN status of the participants. Among those who were hypertensive 11.6% (19) had LEAD whereas 9.6% (273) of those where not hypertensive had LEAD. Among the hypertensive participants LEAD was more prevalent among those of Gr I (13.6%) and Gr II (11.1%) than among those of Gr III (9.7%) the difference between the study groups was not statistically significant (χ^2 = 0.449, df 2; p=0.779). On the other hand among those who were non-hypertensive LEAD was more prevalent among those of Gr I (17.0%) and Gr II (8.9%) than among those of Gr III (3.2%), and the difference between the study groups was statistically significant (χ^2 =104.887, df 2; p<0.001). When adjusted for hypertension status LEAD was found to be about 5.5 (3.8-8.1) and 2.7 (1.8-4.0) times more prevalent (χ^2 =105.905,

df 3; p<0.001) among participants of Gr I and Gr II respectively than among participants of Gr III.

Among the 1760 female participants 5.2% (91) were hypertensive, and 10.0% (176) had LEAD. LEAD was more prevalent among those who were hypertensive (12.1%), than among those who were non hypertensive (9.9%). Among those who were hypertensive, LEAD was more prevalent among those of Gr I (18.4%) and Gr II (8.7%) than among those of Gr III (6.7%) and the difference between the study groups was not statistically significant (Fisher's Exact value=2.201, df 2; p=0.357). Among the non-hypertensive participants, LEAD was more prevalent among those of Gr I (17.0%) and Gr II (9.9%) than among those of Gr III (3.3%) and the difference between the groups was found to be was statistically significant (χ^2 =57.927, df 2; p<0.001). When adjusted for hypertension status LEAD was found to be about 5.6 (3.5-9.2) and 2.9 (1.8-4.9) times more prevalent (χ^2 =63.063, df 3; p<0.001) among participants of Gr I and Gr II respectively than among participants of Gr III.

Among the 1239 male participants 5.9% (73) were hypertensive, and 9.4% (116) had LEAD. LEAD was more prevalent among those who were hypertensive (11.0%), than among those who were non hypertensive (9.3%). Among those who were hypertensive, LEAD was more prevalent among those of Gr III (15.4%) and Gr II (12.5%) than among those of Gr I (7.1%) and the difference between the study groups was not statistically significant (Fisher's Exact value=0.986, df 2; p=0.275). Among the non hypertensive participants, LEAD was more prevalent among those of Gr I (17.1%) and Gr II (7.3%) than among those of Gr III (3.1%) and the difference between the study groups was not statistically significant (χ^2 =48.672, df 2; p<0.001). When adjusted for hypertension status LEAD was found to be about 5.4 (3.0-9.8) and 2.3 (1.2-4.4) times more prevalent (χ^2 =43.826, df 3; p<0.001) among participants of Gr I and Gr II respectively than among participants of Gr III.

Finally when adjusted for hypertension status and gender, LEAD was found to be about 5.6 (3.8-8.1) and 2.7 (1.8-4.0) times more prevalent (χ^2 =106.640, df 4; p<0.001) among participants of Gr I and Gr II respectively than among participants of Gr III.

Table-72: Distribution of respondents by hypertension status, gender, study groups (I & II) and LEAD

HTN	Study group	Gender								
		Male			Female			Combined		
		Total	LEAD		Total	LEAD		Total	LEAD	
			Yes	Prev*		Yes	Prev*		Yes	Prev*
Yes	Gr I	28	2	7.1	38	7	18.4	66	9	13.6
	Gr II	32	4	12.5	30	2	6.7	62	6	9.7
No	Gr I	405	69	17.0	529	90	17.0	934	159	17.0
	Gr II	370	27	7.3	567	56	9.9	937	83	8.9
Total		835	102	12.2	1164	155	13.3	1999	257	12.9
OR (95% CI) adjusted for HTN status	Gr I	2.341 (1.498-3.658)			1.920 (1.355-2.719)			2.066 (1.571-2.716)		
	Gr II	1			1			1		
OR (95% CI) adjusted for HTN status & gender	Gr I	2.074 (1.577-2.728)								
	Gr II	1								

*Prevalence

Among the 1999 study participants 128 (6.4%) were hypertensive, of them only 46.9% (60) were male. Among those who were hypertensive, 11.7% (15) had LEAD, whereas 12.9% (242) of those who were not hypertensive had LEAD. Among the non-hypertensive participants, LEAD was more prevalent among those of Gr I (17.0%) than among those of Gr II (8.9%) and the difference was statistically significant (χ^2 =27.694, df 1; p<0.001). On the other hand among those who were hypertensive, LEAD was more prevalent among those of Gr I (13.6%) than among those of Gr II (9.7%), and the difference between the study groups was not statistically significant (χ^2 = 0.484, df 1; p=0.486). When adjusted for hypertension status, LEAD was found to be about 2.1 (1.6-2.7) times more common (χ^2=28.375, df 2; p<0.001) among members of Gr I than among members of Gr II.

Among the 1164 female participants about 5.8% (68) were hypertensive and 13.3% (155) had LEAD. LEAD was marginally more prevalent among those who were non hypertensive (13.3%), than among those who were hypertensive (13.2%). Among those who were hypertensive though LEAD was more prevalent among those of Gr I (18.4%) than among those of Gr II (6.7%), and the difference between the study groups was not found to be statistically significant (Fisher's Exact Test value =0.280). Among the non

hypertensive participants LEAD was more prevalent among those of Gr I (17.0%) than among those of Gr II (9.9%) and the difference was statistically significant (χ^2 =12.072, df 1; p=0.001). When adjusted for hypertension status LEAD was found to be about 1.9 (1.3-2.7) times more common (χ^2=13.891, df 2; p<0.001) among members of Gr I than among members of Gr II.

Among the 835 male participants about 7.2% (60) were hypertensive and 12.2% (102) had LEAD. LEAD was more prevalent among those who were non hypertensive 12.4% (96) than among those who were hypertensive (10.0%). Among those who were hypertensive, though LEAD was less prevalent among those Gr I (7.1%) than among those of Gr II (12.5%), and the difference between the study groups was not statistically significant (Fisher's Exact value=0.476, df 1; p=0.675). Among the non hypertensive participants LEAD was more prevalent among those Gr I (17.0%) than among those of Gr II (7.3%), and the difference was statistically significant (χ^2 =16.901, df 1; p<0.001). When adjusted for hypertension status LEAD was found to be about 2.3 (1.5-3.6) times more common (χ^2=15.280, df 2; p<0.001) among members of Gr I than among members of Gr II.

When adjusted for diabetic status and gender LEAD was found to be about 2.1 (1.6-2.7) times more prevalent (χ^2=29.164, df 3; p<0.001) among members of Gr I than among members of Gr II.

Table-73: Distribution of respondents by hypertension, gender, excess arsenic exposure and LEAD

HTN	Excess arsenic exposure	Gender								
		Male			Female			Combined		
		Total	LEAD		Total	LEAD		Total	LEAD	
			Yes	*Prev		Yes	*Prev		Yes	*Prev
Yes	Yes	60	6	10.0	68	9	13.2	128	15	11.7
	No	13	2	15.4	23	2	8.7	36	4	11.1
No	Yes	775	96	12.4	1096	146	13.3	1871	242	12.9
	No	391	12	3.1	573	19	3.3	964	31	3.2
Total		1239	116	9.4	1760	176	10.0	2999	292	9.7
OR (95% CI) adjusted for HTN	Yes	3.876 (2.188-6.868)			4.206 (2.636-6.711)			4.068 (2.833-5.841)		
	No	1			1			1		
OR (95% CI) adjusted for HTN & gender	Yes	4.073 (2.837-5.849)								
	No	1								

*Prevalence

Among the 2999 study participants, 164 (5.5%) were hypertensive, of them only 44.5% (73) were male. Among those who were hypertensive 11.6% (19) had LEAD whereas 9.6% (273) of those who were non-hypertensive had LEAD. Among the non-hypertensive participants, LEAD was more prevalent (12.9%) among those with excess arsenic exposure than among with no such exposure (3.2%) and the difference was statistically significant (χ^2 =69.049, df 1; p<0.001). On the other hand among those who were hypertensive, LEAD was more prevalent (11.7%) among those with excess arsenic exposure than among with no such exposure (11.1%), but the difference between the study groups was not statistically significant (χ^2 = 0.317, df 1; p=0.573). When adjusted for hypertension status, LEAD was found to be about 4.1 (2.8-5.8) times more common (χ^2=77.644, df 2; p<0.001) among those having excess arsenic exposure than those having no such exposure

Among the 1760 female participants, about 5.5% (91) were hypertensive and 10.0% (176) had LEAD. LEAD was more prevalent among those who were hypertensive (12.1%), than among those who were non hypertensive (9.9%). Among those who were hypertensive, though LEAD was more prevalent (13.2%) among those with excess arsenic exposure than among with no such exposure (9.9%), and the difference between the study groups was not statistically significant (χ^2=0.333, df 1; p=0.564). Among the

non hypertensive participants, LEAD was more prevalent (13.3%) among those with excess arsenic exposure than among with no such exposure (3.3%), and the difference was statistically significant (χ^2 =42.281, df 1; p<0.001). When adjusted for hypertension status, LEAD was found to be about 4.2 (2.6-6.7) times more common (χ^2=49.124, df 2; p<0.001) among those having excess arsenic exposure than those having no such exposure.

Among the 1239 male participants about 5.9% (73) were hypertensive and 9.4% (116) had LEAD. LEAD was more prevalent among those who were hypertensive (11.0%), than among those who were non hypertensive (9.3%). Among those who were hypertensive though LEAD was less prevalent (10.0%) among those with excess arsenic exposure than among with no such exposure (15.4%), and the difference between the study groups was not statistically significant (χ^2=0.317, df 1; p=0.573). Among the non hypertensive participants LEAD was more prevalent (12.4%) among those with excess arsenic exposure than among with no such exposure (3.1%), and the difference was statistically significant (χ^2 =26.848, df 1; p<0.001). When adjusted for hypertension status LEAD was found to be about 3.9 (2.2-6.9) times more common (χ^2=49.124, df 2; p<0.001) among those having excess arsenic exposure than those having no such exposure.

When adjusted for hypertension status and gender, LEAD was found to be about 4.1 (2.8-5.8) times more prevalent (χ^2=78.127, df 2; p<0.001) among members of Gr I than among members of Gr II.

Table-74: Odds ratio and 95 % CI for LEAD among the study groups adjusted for different morbidity related variables, age and gender.

Gender	Combined			Male			Female		
Study group	Gr III	Gr II	Gr I	Gr III	Gr II	Gr I	Gr III	Gr II	Gr I
Crude	1	2.697 (1.80-4.03)	5.567 (3.82-8.11)	1	2.328 (1.219-4.445)	5.464 (3.026-9.864)	1	2.946 (1.764-4.920)	5.651 (3.472-9.199)
Different morbidity status									
Adjusted for overweight/obesity	1	2.694 (1.803-4.024)	5.549 (3.810-8.082)	1	2.320 (1.215-4.432)	5.414 (2.998-9.777)	1	2.946 (1.764-4.921)	5.651 (3.471-9.202)
Adjusted for anemia	1	2.639 (1.761-3.955)	5.423 (3.705-7.936)	1	2.216 (1.158-4.244)	4.998 (2.746-9.094)	1	2.994 (1.782-5.031)	5.783 (3.524-9.489)
Adjusted for pedal edema	1	2.697 (1.805-4.028)	5.615 (3.856-8.176)	1	2.328 (1.219-4.445)	5.540 (3.068-10.003)	1	2.946 (1.764-4.920)	5.675 (3.486-9.238)
Adjusted for amputation	1	2.697 (1.805-4.028)	5.468 (3.753-7.966)	1	2.328 (1.219-4.445)	5.233 (2.893-9.466)	1	2.946 (1.764-4.920)	5.651 (3.472-9.199)
Adjusted for DM status	1	2.659 (1.779-3.974)	5.471 (3.755-7.972)	1	2.303 (1.205-4.400)	5.369 (2.971-9.702)	1	2.901 (1.736-4.847)	5.555 (3.410-9.050)
Adjusted for HTN	1	2.691 (1.801-4.021)	5.555 (3.813-8.091)	1	2.319 (1.213-4.433)	5.450 (3.017-9.844)	1	2.943 (1.762-4.915)	5.636 (3.461-9.178)
Adjusted for DM status, age and gender	1	2.834 (1.889-4.251)	6.016 (4.109-8.806)						
Adjusted for HTN status, age and gender	1	2.875 (1.917-4.311)	6.107 (4.174-8.935)						
Adjusted for age, overweight/obesity, DM status and HTN	1	2.837 (1.891-4.256)	5.950 (4.064-8.709)	1	2.558 (1.327-4.931)	6.245 (3.417-11.414)	1	3.015 (1.797-5.058)	5.863 (3.582-9.596)
Adjusted for age, gender, overweight/obesity, DM status and HTN	1	2.835 (1.890-4.254	5.995 (4.094-8.778)						
Adjusted for morbidity related variables and age	1	2.788 (1.851-4.200)	5.765 (3.913-8.494)	1	2.461 (1.272-4.761)	5.678 (3.072-10.496)	1	3.085 (1.825-5.215)	6.068 (3.674-10.023)
Adjusted for morbidity related variables, age and gender	1	2.822 (1.873-4.253)	5.896 (3.998-8.695)						

A series of logistic regression analyses were undertaken to determine the OR (95% CI) of LEAD in different study groups adjusted for the different morbidity related variables (overweight/obesity, anemia, pedal edema, amputation, DM status and HTN) separately as well as together along with age and gender.

The OR for LEAD among females when adjusted for overweight/obesity was found to be 5.651 and 2.946 among Gr I and Gr II participants respectively compared to those belonging to Gr III (χ^2 =62.992, df 3; p<0.001). And among males the LEAD was found to be 2.32 and 5.414 times more common among participants of Gr II and Gr I respectively than that of among participants of Gr III (χ^2 =45.380, df 3; p<0.001). The overweight/obesity adjusted OR among females remained the same as the crude OR for female, and among males the crude and adjusted ORs were almost similar.

The ORs for LEAD among the different study groups when adjusted for anemia, pedal edema, amputation, diabetes and hypertension showed little or no deviation from the crude ORs for different study groups in both the sexes.

When adjusted for the differences in age, gender and DM status between the study groups it was found that LEAD was about 6.0 and 2.8 times more prevalent (χ^2=173.966, df 5; p<0.001) among participants of Gr I and Gr II respectively than among participants of Gr III. Again when adjusted for the differences in age, gender and HTN status between the study groups it was found that LEAD was about 6.1 and 2.9 times more prevalent (χ^2=172.278, df 5; p<0.001) among participants of Gr I and Gr II respectively than among participants of Gr III.

When separately adjusted for age, overweight/obesity, DM status and HTN status, and again for age, gender, overweight/obesity, DM status and HTN a slight increase in the ORs was observed.

When separately adjusted for all of the morbidity variables together with age LEAD was found to be 6.068 and 3.085 times more prevalent amongst females (χ^2 =92.007, df 6; p<0.001) and, 5.678 and 2.461 more prevalent among males (χ^2 =100.385, df 10; p<0.001) of Gr I and Gr II than amongst those of Gr III.

Finally when adjusted for all of the morbidity variables together with age and gender LEAD was found to be 5.869 and 2.822 times higher among participants of Gr I and Gr II than amongst those of Gr III (χ^2 =187.459, df 12; p<0.001).

4.5 LEAD AMONG THE STUDY GROUPS ADJUSTED FOR DIFFERENT STUDY VARIABLES.

Table-75: Odds ratio and 95 % CI of LEAD among the study groups adjusted for different study variables.

Study group	GR I, II and III			GR I and II		Excess arsenic exposure	
	Gr III	Gr II	Gr I	Gr II	Gr I	No	Yes
Crude OR (95%CI)	1	2.697 (1.80-4.03)	5.567 (3.82-8.11)	1	2.065 (1.570-2.714)	1	4.068 (2.833-5.841)
OR (95%CI) adjusted for							
All socio-demographic variables	1	3.088 (2.037-4.682)	6.263 (4.240-9.251)	1	2.052 (1.546-2.724)	1	4.653 (3.195-6.777)
All food habit related variables, age & gender	1	3.114 (2.054-4.721)	6.766 (4.557-10.047)	1	2.137 (1.614-2.828)	1	4.758 (3.261-6.942)
Tobacco consumption, age & gender	1	2.706 (1.795-4.081)	5.722 (3.879-8.441)	1	2.089 (1.582-2.757)	1	4.087 (2.818-5.927)
All morbidity related variables, age and gender	1	2.822 (1.873-4.253)	5.896 (3.998-8.695)	1	2.069 (1.567-2.732)	1	4.229 (2.915-6.135)
All study variables	1	3.013 (1.939-4.680)	6.210 (4.081-9.449)	1	2.049 (1.532-2.742)	1	4.499 (3.008-6.729)

When adjusted for all socio-demographic variables (age, gender, household size, education level, occupation, housing type, annual household income, and household possession of agricultural land, radio, bicycle, television and motorcycle) LEAD was found to be 6.3 (4.2-9.2) times and 3.1(2.0-4.7) times more prevalent among members of Gr I and Gr II respectively than among members of Gr III (χ^2= 194.228, df 18; p<0.001). LEAD was found to be 2.1 (1.5-2.7) times more prevalent (χ^2= 91.025, df 17; p<0.001) among those having arsenicosis and excess arsenic exposure (Gr I) than amongst those having excess arsenic exposure but not having arsenicosis (Gr II). Again when presence or absence of arsenicosis was ignored, LEAD was found to be 4.6 (3.2-6.8) times more prevalent (χ^2=169.360, df 17; p<0.001) among those having excess arsenic exposure compared to those not having exposure to excess arsenic.

When adjusted for all food habits related variables (staple food type, numbers of days of vegetable intake per week, number of days of fish/meat/egg intake per week, numbers of days of milk or milk products intake per week and cooking oil) together with age and gender LEAD was found to be 6.8 (4.6-10.0) and about 3.1 (2.0-4.7) more prevalent (χ^2=177.437, df 9; p<0.001) among participants of Gr I and Gr II respectively compared to those belonging to Gr III. LEAD was found to be 2.1 (1.6-2.8) times more prevalent (χ^2= 70.539, df 8; p<0.001) among those having arsenicosis and excess arsenic exposure (Gr I) than amongst those having excess arsenic exposure but not having arsenicosis (Gr II). Again when presence or absence of arsenicosis was ignored, LEAD was found to be 4.7 (3.3-6.9) times more prevalent (χ^2=146.922, df 8; p<0.001) among those having excess arsenic exposure compared to those not having exposure to excess arsenic.

When adjusted for all tobacco consumption related variables (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco) along with age and gender, LEAD was found to be about 5.7 (3.9-8.7) and 2.7 (1.8-4.2) times ore prevalent (χ^2= 181.535, df 9; p<0.001) among participants of Gr I and Gr II respectively compared to those belonging to Gr III. LEAD was found to be 2.1 (1.6-2.8) times more prevalent (χ^2= 72.909, df 8; p<0.001) among those having arsenicosis and excess arsenic exposure (Gr I) than amongst those having excess arsenic exposure but not having arsenicosis (Gr II). Again when presence or absence of arsenicosis was ignored, LEAD was found to be 4.7 (3.3-6.9) times more prevalent (χ^2=152.699, df 8; p<0.001) among

those having excess arsenic exposure compared to those not having exposure to excess arsenic.

When adjusted for the different morbidity related variables (overweight/obesity, anemia, pedal edema, amputation, DM status and HTN) along with age and gender, LEAD was found to be about 5.9 (4.0-8.7) and 2.8 (1.9-4.2) times higher (χ^2 =187.459, df 12; p<0.001) among participants of Gr I and Gr II respectively compared to those belonging to Gr III . LEAD was found to be 2.1 (1.6-2.7) times more prevalent (χ^2= 82.217, df 11; p<0.001) among those having arsenicosis and excess arsenic exposure (Gr I) than amongst those having excess arsenic exposure but not having arsenicosis (Gr II). Again when presence or absence of arsenicosis was ignored, LEAD was found to be 4.7 (3.3-6.9) times more prevalent (χ^2=159.660, df 11; p<0.001) among those having excess arsenic exposure compared to those not having exposure to excess arsenic.

Finally when adjusted for all socio-demographic variables, all food habits related variables, all tobacco consumption related variables and all morbidity related together, LEAD was found to be about 6.2 (4.1-9.4) and 3.0 (1.9-4.7) times higher (χ^2 =228.005, df 36; p<0.001) among participants of Gr I and Gr II respectively compared to those belonging to Gr III . LEAD was found to be 2.0 (1.5-2.7) times more prevalent (χ^2= 120.099, df 35; p<0.001) among those having arsenicosis and excess arsenic exposure (Gr I) than amongst those having excess arsenic exposure but not having arsenicosis (Gr II). Again when presence or absence of arsenicosis was ignored, LEAD was found to be 4.5 (3.0-6.7) times more prevalent (χ^2=203.348, df 35; p<0.001) among those having excess arsenic exposure compared to those not having exposure to excess arsenic.

4.6 DOSE EFFECT RELATIONSHIP

Table-76: Distribution of respondents by cumulative arsenic exposure category and LEAD

Arsenic exposure category (mg-year/L)	Combined				Male				Female			
	Total (%)	LEAD			Total	LEAD				LEAD		
		Yes	Prev* (%)	OR (95% CI)		Yes	Prev* (%)	OR (95% CI)	Total	Yes	Prev* (%)	OR (95% CI)
<1	790 (26.3)	16	2.0	1	308	8	2.6	1	482	8	1.7	1
1-4	242 (8.1)	19	7.9	4.12 (2.09-8.15)	100	6	6.0	2.39 (0.81-7.07)	142	13	9.2	5.97 (2.42-14.71)
5-9	1065 (35.5)	98	9.2	4.90 (2.87-8.39)	330	21	6.4	2.55 (1.11-5.84)	735	77	10.5	6.93 (3.32-14.50)
≥10	902 (30.1)	159	17.6	10.35 (6.13-17.48)	501	81	16.2	7.23 (3.45-15.18)	401	78	19.5	14.31 (6.82-30.03)
Total	2999	292	9.7	χ^2 = 130.86, df 3; p<0.001	1239	116	9.4	χ^2 = 48.78, df 3; p<0.001	1760	176	10.0	χ^2 =77.35, df 3; p<0.001
Significance	χ^2 = 118.68, df 3; p<0.001				χ^2 = 51.02, df 3; p<0.001				χ^2 = 87.70, df 3; p<0.001			

*Prevalence

When arsenic exposure was examined in terms of cumulative 30.1% (902) and 35.5% of the total 2999 participants were found to have an exposure of that fell in the categories of ≥10 mg-yr/L and 5-9 mg-yr/L respectively. Small proportion of participants (8.1%) had a cumulative arsenic exposure in the category of 1-4 mg-yr/L and another 26.3% had a cumulative arsenic exposure in the category of <1 mg-yr/L.

Increasing prevalence of LEAD was found with increasing cumulative arsenic exposure (χ^2 = 118.683, df 3; p<0.001). In the lowest cumulative arsenic exposure category (<1 mg-yr/L) the prevalence of LEAD was found to be 2.0% and a prevalence of 17.6% was found in the highest cumulative arsenic exposure category (≥10 mg-yr/L). Increasing prevalence of LEAD with increasing cumulative arsenic exposure was found observed both among males (p<0.001) and females (p<0.001).

Fig No-31: Prevalence of LEAD by gender and arsenic exposure category

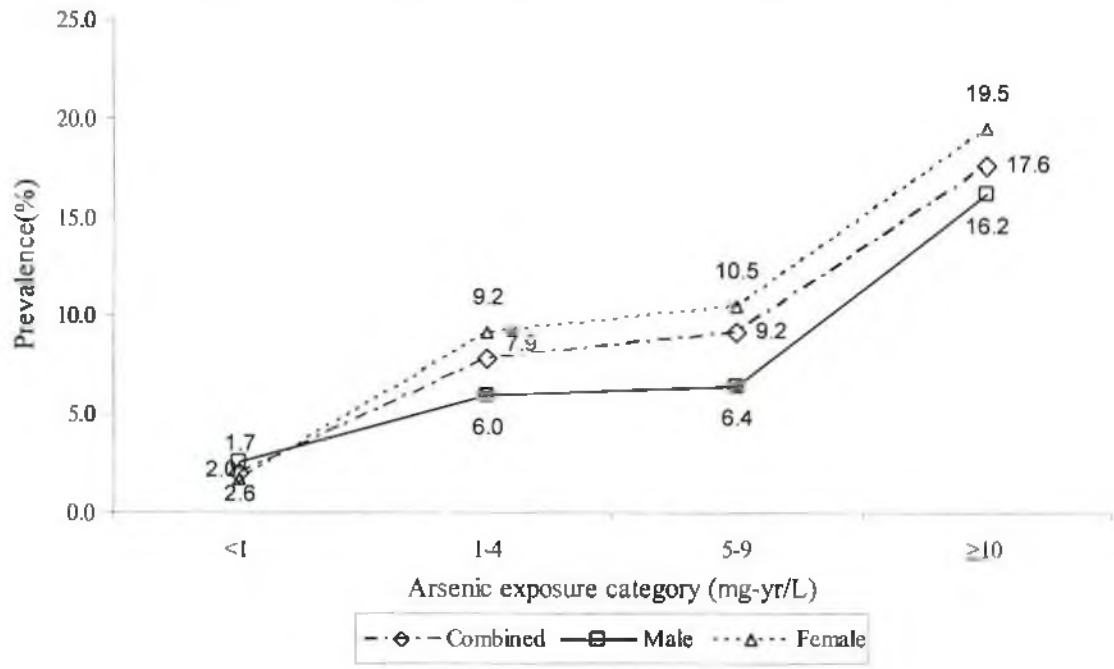
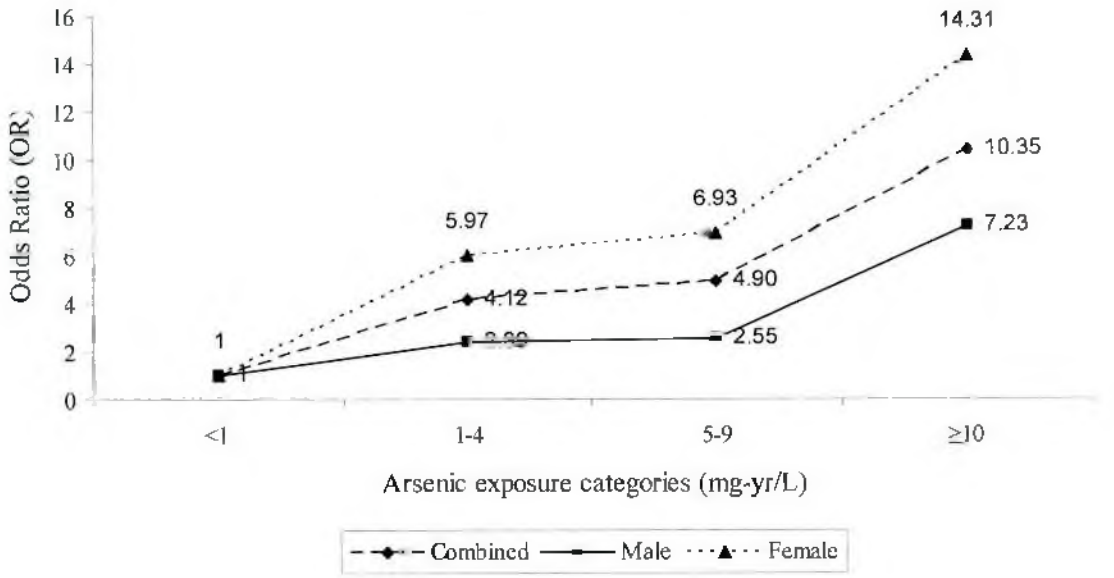


Fig No-32 : Odds ratio of LEAD among study participants in different arsenic exposure categories (mg-yr/L)



LEAD was found to be 4.1, 4.9, and 10.3 times higher among participants having cumulative arsenic exposure in the categories of 1-4 mg-yr/L, 5-9 mg-yr/L and ≥10 mg-yr/L respectively in comparison to those having cumulative arsenic exposure in the category of <1 mg-yr/L (χ^2 = 130.864, df 3; p<0.001).

Among males LEAD was found to be about 2.4, 2.5, and 7.2 times higher among participants having cumulative arsenic exposure in the categories of 1-4 mg-yr/L, 5-9 mg-yr/L and ≥10 mg-yr/L respectively in comparison to those having cumulative arsenic exposure in the category of <1 mg-yr/L (χ^2 =48.78, df 3; p<0.001). Among the female participants, LEAD was found to be about 6.0, 6.9 and 14.3 times higher among those having cumulative arsenic exposure in the categories of 1-4 mg-yr/L, 5-9 mg-yr/L and ≥10 mg-yr/L respectively in comparison to those having cumulative arsenic exposure in the category of <1 mg-yr/L (χ^2 =77.35, df 3; p<0.001).

Table-77: Odds ratio and 95 % CI of LEAD among the cumulative arsenic exposure category (mg-year/L) adjusted for different study variables.

OR (95% CI) for LEAD	Arsenic exposure category(mg-year/L)				Significance
	<1	1-4	5-9	≥10	
Crude OR	1	4.122 (2.085-8.148)	4.903 (2.866-8.386)	10.352 (6.131-17.479)	χ^2 = 130.864, df 3; p<0.001
OR (95% CI) for LEAD adjusted for					
All socio-demographic	1	2.885 (1.430-5.820)	5.741 (3.313-9.949)	8.773 (5.101-15.089)	χ^2 = 188.107, df 19; p<0.001
All food habits, age & gender	1	2.910 (1.447-5.850)	5.925 (3.420-10.264)	9.096 (5.276-15.682)	χ^2 = 166.446, df 10; p<0.001
Tobacco consumption, age & gender	1	2.985 (1.483-6.009)	5.121 (2.971-8.824)	7.809 (4.560-13.372)	χ^2 = 172.037, df 10; p<0.001
Age, gender, tobacco consumption, overweight/obesity, DM & HTN	1	2.966 (1.472- 5.978)	5.036 (2.920-8.684)	7.688 (4.485-13.179)	χ^2 = 86.068, df 10; p<0.001
All morbidity related variables (excluding skin manifestation) age & gender	1	2.869 (1.425- 5.776)	5.285 (3.066-9.111)	8.009 (4.663-13.758)	χ^2 = 84.469, df 10; p<0.001
All morbidity related variables (includes skin manifestation) age & gender	1	2.715 (1.347-5.470)	3.926 (2.227-6.921)	5.189 (2.919-9.225)	χ^2 = 261.323, df 15; p<0.001
Age, gender, tobacco consumption, overweight/obesity, DM,HTN & skin manifestations (melanosis ± keratosis)	1	2.808 (1.392- 5.664)	3.711 (2.104-6.548)	4.889 (2.754-8.679)	χ^2 = 37.232, df 3; p<0.001
All study variables except skin manifestation	1	3.037 (1.500-6.147)	5.745 (3.262-10.119)	8.619 (4.920-15.097)	χ^2 = 222.068, df 37; p<0.001
All study variables	1	2.859 (1.411-5.793)	4.329 (2.401-7.804)	5.659 (3.118-10.269)	χ^2 = 301.595, df 39; p<0.001

A series of logistic regression analyses were undertaken using presence or absence of LEAD as the binary dependent variable to determine the OR (95%CI) LEAD in different cumulative arsenic exposure categories adjusted for different study variables. The results are presented in Table 77.

Adjustments were done for all socio-demographic variables (age; gender; educational status; occupation; family ownership of agricultural land, radio television, bicycle, motorcycle; household size housing type; and annual household income); food habits (staple food type, numbers of days of vegetable intake per week, number of days of fish/meat/egg intake per week, numbers of days of milk or milk products intake per week and cooking oil), age and gender; tobacco consumption (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco), age and gender; all morbidity related variables [overweight or obesity, anaemia, pedal oedema, amputation, hypertension, diabetes, and skin manifestation (melanosis, keratosis)] age and gender; all study variables except skin manifestation; and finally all study variables.

In all of the scenarios considered for adjustment, LEAD was found to be more prevalent with increasing cumulative arsenic exposure ($p<0.05$).

Table No-78: Summary table-comparability of the study groups and risk difference for LEAD in reference to excess arsenic exposure.

Comparability of the study groups		Significance	
Comparability of study groups in terms of sociodemographic characteristics	gender, age, schooling, occupation, household size, household income, and type of housing	p>0.05	Non significant
	household possession of: agricultural land; television, radio or 2-in-1, bicycle and motorcycle.	p<0.05	Significant
Comparability of the groups in terms of selected risk factors	nutritional status (BMI) and smoking status (male).	p > 0.05	Non significant
	diabetic and blood pressure status	p<0.05	Significant

Risk difference for LEAD in reference to excess arsenic exposure.		Study groups		
		Gr-I	Gr-II	Gr-III
Prevalence (%) of LEAD	Crude	16.8	8.9	3.5
	Adjusted for age and gender	16.8	9.2	3.3
Odds ratio (OR) and 95% CI of LEAD among the study groups adjusted for selected risk factors	Crude	1	2.80 (1.80-4.03)	5.67 (3.82-8.11)
	Adjusted for age	1	2.57 (1.34-4.95)	6.05 (4.14-8.85)
	Adjusted for gender	1	2.70 (1.81-4.03)	5.59 (3.84-8.14)
	Adjusted for age gender, tobacco consumption, & duration of tobacco consumption.	1	2.71 (1.80-4.08)	5.72 (3.88-8.44)
	Adjusted for overweight /obesity	1	2.69 (1.80-4.02)	5.55 (3.81-8.08)
	Adjusted for DM status	1	2.66 (1.78-3.97)	5.47 (3.76-7.97)
	Adjusted for HTN	1	2.69 (1.80-4.02)	5.56 (3.81-8.09)
	Adjusted for age, gender, overweight/obesity, DM status and HTN	1	2.84 (1.89-4.25)	6.00 (4.09-8.78)

Odds ratio (OR) and 95 % CI of LEAD among the cumulative arsenic exposure categories adjusted for different study variables.	Variable	Cumulative arsenic exposure category (mg-year/L)			
		<1	1-4	5-9	≥10
	Crude OR	1	4.12 (2.09-8.15)	4.90 (2.87-8.39)	10.35 (6.13-17.48)
	All socio-demographic	1	2.89 (1.43-5.82)	5.74 (3.31-9.95)	8.77 (5.10-15.09)
	Tobacco consumption, age & gender	1	2.99 (1.48-6.01)	5.12 (2.97-8.82)	7.81 (4.56-13.37)
	Age, gender, tobacco consumption, overweight /obesity, DM & HTN	1	2.97 (1.47-5.98)	5.04 (2.92-8.68)	7.69 (4.49-13.18)
	Age, gender, tobacco consumption, overweight /obesity, DM,HTN & skin manifestations (melanosis ± keratosis)	1	2.81 (1.39-5.67)	3.71 (2.10-6.55)	4.89 (2.75-8.68)

CHAPTER V

DISCUSSION

5.1 INTRODUCTION

Arsenic, the 20th most abundant element in the earth's crust is found ubiquitously in nature. Both organic and inorganic forms of arsenic may occur in the soil, air, water, food and some medications. Inorganic forms of arsenic, usually present in tubewell water are much more toxic than organic forms. Chronic exposure with low dose of arsenic from drinking water, coal burning, industrial sources or food has been reported from different parts of the world[32-37,41,70,73,74,77,78, 83-102,142,146,151-155,392-394]. For humans not occupationally exposed, the most significant pathway of exposure is the oral intake of arsenic through food, beverages and drinking water[32,33,36,37,41,79]. In areas with elevated concentrations of arsenic in drinking-water, this source makes a significant contribution to the total intake of inorganic arsenic, while other routes of intakes of arsenic (intranasal and dermal) are of minor importance in comparison to the oral route[33]. In areas where drinking-water contains arsenic >0.05 mg/L, water may be the major source of inorganic arsenic. Inhalation would add about 1 μg of arsenic per day from airborne particulates and approximately 6 μg/day may be inhaled from 20 cigarettes[32,37]. Chronic exposure with low dose of arsenic has been found can cause skin lesions, neurological defects, atherosclerosis and cancer[32,33,36,37,41,79,392-398]. Considering the toxicological importance of arsenic present in drinking water, World Health Organization in 1958 had established 0.2 mg/L as an allowable concentration in drinking water, in 1963 a stricter concentration of 0.05 mg/L was set as a new standard and in 1993 a further stricter standard of 0.01 mg/L was suggested as a provisional guideline value[141,142, 148]. The problem of arsenic exposure from drinking water can also be found in different regions of the world, including Argentina, Australia, Chile, China, Hungary, Mexico, Peru, Taiwan, Thailand and the United States of America[32-37,48,139,141,142, 392-394]. Currently, Bangladesh and West Bengal-India have the most serious problem of groundwater contamination with arsenic in the world [48,53,144,396].

Though arsenic is considered a potent human hazard because of its neoplastic outcomes, increasing epidemiological evidence suggests that there is also a link between arsenic exposure and risk of vascular diseases related to atherosclerosis. Based on available evidence it has been suggested that arsenic exposure is an independent risk factor for atherosclerosis[35,40,45,49,268,275,380,395,397-402]. Arsenic has been found in epidemiologic

studies to be associated with hypertension[64,240] and diabetes mellitus[65,150,239,339-341,403-405], all of these could trigger, predispose, or aggravate the process of atherosclerosis. Arsenic is considered a major risk factor for a peripheral artery disease characterized by severe arteriosclerosis and subsequent gangrene of affected extremities, often called as "blackfoot" disease (BFD). The epidemiological evidence for an association between BFD and arsenic is obtained from ecological, case-control studies, and from the co-occurrence of BFD with arsenic-dependent cancers[45,47,49239,266-268]. In addition to that in Taiwan, an increased prevalence of peripheral vascular disease has also been documented among inhabitants with long-term exposure to drinking water contaminated with high level of arsenic in Chile and Mexico[32,37]. Though mechanisms of arsenic-induced atherosclerosis still remain to be elucidated, available in vitro evidences can possibly explain the atherogenic effect of arsenic. The atherogenicity of arsenic could be associated with hypercoagulability, endothelial injury, smooth muscle cell proliferation, somatic mutation, oxidative stress, and apoptosis[233,234,321,322,325,330,335,394,406]. A recent study treating $ApoE^{-/-}/LDLr^{-/-}$ mice with sodium arsenite in drinking water for 18 weeks has successfully induced a significant increase in atherosclerotic plaques in the innominate artery compared to controls[318]. And this animal model provided evidence for biological plausibility of arsenic-induced atherosclerosis observed in humans in epidemiologic studies.

Based on the reversibility criterion, the gradual decline in PVD mortality after cessation of consumption of high-arsenic artesian well water as observed among residents in the arseniasis-endemic areas in Taiwan from 1971 to 2003[407] strengthens the likelihood of the association that exists between arsenic exposure and development of PVD.

5.2 CONTEXT OF THE PRESENT STUDY

Arsenic contamination of groundwater in Bangladesh was initially reported in 1993, followed by official reporting of arsenicosis in 1994[52]. It is estimated that more than 95% of the 120 million people in Bangladesh drink tubewell water and than 50 million people are estimated to be ingesting contaminated water in Bangladesh [55,83,146,408]. Some 27% of shallow (<150 m deep) wells in Bangladesh contain more than 50 mg/L arsenic[55,408]. In Bangladesh concentrations of arsenic in groundwater as high as 4.0 mg/L has been reported from Chatkhil of Noakhali district[55,163,408]. The worst-affected area is in the SE

of Bangladesh where in some districts more than 90% of wells are affected[55,227]. Arsenic contamination of groundwater tapped through tubewells had been reported from 60 out of 64 districts of Bangladesh [54,56,84]. In surveys covering 57482 villages of 2934 unions in 270 upazillas, 29.12% of the 4946933 tubewells were found to be arsenic contaminated. And among 66034962 individuals residing in 12001665 households of these upazillas a total of 38430 individuals had been reported as suspected arsenicosis patients[227]. Many of the health problems known to be related to arsenic exposure has been reported in Bangladesh[39,52-69], studies focusing on the health effects of arsenic in Bangladesh has also reported the occurrence of gangrene an extreme manifestations of LEAD [53,54,57,58]. Among them 400 cases had been listed in Alamdanga of Chuadanga (NIPSOM, World Vision, DGHS), 568 cases in Shahrasti of Chandpur (DGHS, NIPSOM, ICDDRB), and 1791 cases in Laksham of Comilla (ICDDRB, DGHS, NIPSOM). Individuals with excess arsenic exposure (>0.05 mg/L) both with and without arsenicosis, as well as individuals without excess arsenic exposure (<0.05mg/L) are traceable in Bangladesh. Thus the context in Bangladesh offers a unique opportunity to study arsenic related health effects.

The cross-sectional comparative study was designed with comparison group(s) to explore if exposure to excess arsenic through drinking water was posing any risk difference for LEAD in Bangladesh. In this study, participants were recruited in three groups. There was a reference group (Gr III) which included participants without excess arsenic exposure (drinking water arsenic content <0.05 mg/L); and another two groups both having excess arsenic exposure (drinking water arsenic content >0.05 mg/L), of which participants of one group had arsenicosis (Gr I) while participants in the other group (Gr II) did not have the dermal signs of arsenicosis. Participants for Gr I and Gr II had been selected from Alamdanga, Shahrasti and Lakhsam upazillas while participants for Gr III had been selected from Pakundia upazilla. Based on group specific inclusion and exclusion criteria and informed consent, 1000 participants were selected for each of the study groups. Initially from the lists of clinically confirmed arsenicosis cases in each of the above upazillas, eligible and consenting individuals aged 30-60 years of age were recruited as participants for Gr I. Individuals in the household, from which a participant for Gr I had been included eligible and consenting individual(s) were selected as participants for Gr II, in case when such eligible individual was found in that household

the adjacent household(s) were explored to recruit an eligible consenting individual for Gr II. The Gr I participants had signs of arsenicosis (melanosis and/or keratosis) and history of consuming or having had consumed drinking water from sources identified as having excess arsenic (>0.05 mg/L). The Gr II consisted of participants who had excess arsenic exposure but did not have arsenicosis had been selected. During the process of selection of participants for groups II and III, gender and age (±2 years) matching for participants recruited for Gr I was attempted.

Besides, LEAD (ABI≤0.9) and arsenic consumption, variables also considered included socio-demographic variables (age, gender, household size, education level, occupation, housing type, annual household income, and household possession of agricultural land, radio, bicycle, television and motorcycle), food habits related variables (staple food type, numbers of days of vegetable intake per week, number of days of fish/meat/egg intake per week, numbers of days of milk or milk products intake per week and cooking oil), tobacco consumption related variables (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco) different morbidity related variables (overweight/obesity, anemia, pedal edema, amputation, DM status and HTN).

In this study, the prevalence of lower extremity arterial disease (LEAD) amongst those exposed to excess arsenic and having signs of arsenicosis; amongst those exposed to excess arsenic and not having signs of arsenicosis; and amongst the population not having been exposed to excess arsenic was determined and compared.

5.3 COMPARABILITY OF THE GROUPS

Comparability in terms of sociodemographic characteristics

Males constituted 43.3%, 40.3% and 40.4% of the study participants of Gr-I, Gr-II and Gr-III respectively (table-17). The mean age of the respondents of GR-I, GR-II and GR-III were 40.2±7.6 (39.8-40.7), 40.2±7.6 (39.7-40.7) and 40.8±7.9 (40.3-41.3) years respectively (table-19). Majority (98.6%) of study participants were Muslim while the rest (1.4%) were Hindus. The proportion of Hindus in the different study groups were 1.6%, 1.0% and 1.7% in GR-I, GR-II and GR III respectively (table-21). The mean household size for GR-I, GR-II and GR-III were 6.45±1.179, 6.44±1.183 and 6.55±1.020 respectively (table-23). The number of participants reporting of having

received some level of schooling was consistently high in all the groups (table-24). Most of the male participants (≥54.3%) in the all the study groups were involved in agriculture or farming. Business-shop keeping-hawker was the second largest occupational group among male participants of all the study groups ((15.9~21.8%). Small proportions (2.0-2.1%) of the participants in the different groups were either pensioners or had given up their occupation (retired).And females in groups were mostly (Gr I- 97.9%, Gr II 98.8% and Gr III- 98.3%) found to be involved in household work (table-25).

Almost 35% of the study participants were from household with no agricultural land and only 25.5% were from households having 3 to 25 bighas of land (table-26). Higher proportion (40%) of participants in Group II had no cultivatable land compared to that for other groups (33.3% in Gr-I and 30.7% for Gr-III). Fewer (20.8%) participants of Gr-II were from households having 3-25 bighas of agricultural land compared to participants of the other groups (28.7% for Gr-I and 27.1% for GR-III). Highest proportion of the study participants in all the groups (Gr I- 63.9%, Gr II- 62.3% and Gr III-68.3%) lived in households having kacha floor, mud walls and tin or tally roof (table-27). Only 0.8% of the study participants lived in houses having kacha floor, thatched walls and thatched roof (Gr I- 0.8%, Gr II- 1% and Gr III- 0.7%). More over less than 1% lived in pucca household (Gr I- 0.98%, Gr II- 1% and Gr III- 0.8%). Approximately 35% of the study participants (40.7% in Gr I, 32.0% in Gr II and 31.5% in Gr III) had a television in the household (table-28). Radio or 2 in1 was possessed by 29.1%, 25.2% and 39.8% households of the participants of Gr-I, Gr-II and Gr-III respectively. Bicycle was possessed by 39.3%, 37.1% and 56.0% households of participants of Gr-I, Gr-II and Gr-III respectively. Among Gr-I, Gr-II and Gr-III participants 8.7%, 5.2% and 9.0% respectively had a motorcycle in the household. The mean annual household income of participants of Gr-I, Gr-II and Gr-III were Tk 92914.50/- (±Tk42414.13/-), Tk 91415.21/- (±Tk44100.45/-) and Tk 92145.60/- (±Tk51244.06/-) respectively (table-29).

The study groups were statistically similar ($p>0.05$) in terms of gender, age, schooling, occupation, household size, household income, and type of house in which they lived. But the groups were found to be statistically different ($p<0.05$) in terms of household possession of agricultural land; household possession of television, radio or 2 in1, bicycle and motorcycle.

Comparability of the groups in terms of selected risk factors

Among all the participants most (91.8%) had normal BMI, 7.2% were overweight and a few (1.1%) were obese (table-table-37). Obesity was found to be less common among participants of Gr I (0.8%) than among participants of the other two groups (1.2%). None of the female participants in any of the groups were either current smokers or had smoked in the past. Among the males 43.5% were current smokers, 50.5% had never smoked and the remaining 6.0% were past smokers (table-35). Based on fasting capillary blood glucose level of ≥6.1 mmol/L and history of taking medication for diabetes mellitus, 6.8%, 5.9% and 3.1% participants of Gr I, Gr II and Gr III respectively were found to be diabetic (table-38). Based on history of currently being on antihypertensive medication and blood pressure measurements, hypertension (table-39) was found to be more common amongst participants of Gr I (6.6%) than among participants of Gr II (6.3%) and Gr III (3.6%). Prehypertension was also more common among participants of Gr I (34.5%) than among participants of either Gr II (32.2%) or Gr III (23.1%).

The study groups were statistically similar ($p > 0.05$) in terms nutritional status (BMI) and smoking status (male). But were found to be statistically different ($p<0.05$) in terms of diabetic and blood pressure status of the participants.

Arsenic level in current and past drinking water sources

The arsenic level in the current drinking water source (table-40) of the participants varied from 0~0.640 mg/L for those of Gr I, 0~0.560 mg/L for those of Gr II and 0.002~0.045 mg/L for those of Gr III. The mean arsenic levels (mg/L) of the current water source of the participants of Gr I, Gr II and Gr III were 0.251 (±0.131), 0.259 (±0.081) and 0.002 (±0.007) respectively. Moreover the groups were significantly different ($p<0.05$) in terms of arsenic content of the water of their current drinking water source.

The arsenic level of the past drinking water source (table-41) of the participants of Gr I was 0.100~0.641 mg/L and the mean level was 0.289 (±0.131) mg/L. That for participants of Gr II was 0.004~0.564 mg/L and the mean level was 0.250 (±0.135). While the arsenic level of the past water source of the participants of Gr III was 0.004~0.047 mg/L and the mean level was 0.021 (±0.007). The groups were significantly different ($p<0.05$) in terms of arsenic content of the water of their current drinking water source.

Ankle brachial index (ABI)

Ankle brachial index being a highly specific and sensitive objective tool for detecting ABI in epidemiological studies[1-14,16-18,20,23-26,228,229] was used to detect LEAD, as in the current study. Individual having an ABI of ≤0.9 was considered as having LEAD[1-3,6-8,10,12,20,21,24,27-29,228,229, 246,259] and those having an ABI of >1.3 was considered as having an incompressible artery [2].

The mean ABI among the all of the study participants was found to be 0.99 (± 0.09). The mean ABI for participants in Gr I, Gr II and Gr III was found to be 0.97 (±0.09), 0.98 (±0.09) and 1.01 (±0.09) respectively. The mean ABI for Gr I and Gr II was significantly lower ($p<0.05$) than the mean ABI for Gr III (table-43).

Of the total 3000 study participants in three groups 1 participant in Gr II had an ABI of >1.30, and hence was not included in all subsequent analyses.

5.4 LEAD IN NORMAL POPULATION (REFERENCE GROUP)

As the epidemiology of LEAD in Bangladesh is not available the analysis encompassing participants of Gr III was carried out to compare selected factors. An increasing trend in LEAD prevalence with increasing age was observed (fig-28), and this trend was found to be statistically significant ($\chi^2=41.937$, df=2; $p<0.001$). Studies conducted elsewhere had also demonstrated that the incidence and prevalence of LEAD are strongly age dependent and that the prevalence increases with age[1-5,7-10,15,16,20,2324,25,231,246,,248].

Traditional risk factors

Age & gender

Age adjusted prevalence of LEAD (table-48) among the participants of the reference group was found to be 3.3% (2.7-4.0), that for male was found to be 3.2% (95% CI 2.2-4.2) while that for females was 3.4% (2.5-4.2). And the age and gender adjusted prevalence of LEAD (table-48) among population without excess arsenic exposure was found to be 3.3% (2.650-3.926). In populations younger that 60 years of age an approximate prevalence of 3% had been reported[9,16], another study had reported a prevalence of 2.5% at ages of 40 to 59 years[7]. It appears that the prevalence of LEAD in the population of Bangladesh without excess arsenic exposure was almost similar to that prevailing elsewhere.

Smoking, diabetes & HTN

LEAD has been reported to been reported to be strongly associated with hypertension[1,2,5,7,17,21,23,247,250]. And 50-90% of individuals with LEAD had been reported to have hypertension[249,250]. Based on a PAD (LEAD) diagnosis of an ABI <0.9, Cardiovascular Health Study (CHS) [252,253] had revealed that the relative risk (RR) for PAD was 4.05 for diabetes and 2.55 for current smoking status. In the reference population of this study the likelihood of an individual having LEAD was found to be higher among those who were ever smoker (OR 1.6) among diabetics (OR 3.1) or those who were hypertensive (OR 3.8). In contrast, Stoffers HE et al (1997)[254] had reported the likelihood of an individual having LEAD (ABI<0.9) was increased by having diabetes (OR 2.5) or being a smoker (OR 1.6).

5.5 ARSENIC EXPOSURE AND LEAD

Study groups, Gender, Age & LEAD

The study had revealed that those having arsenicosis (Gr I) had a significantly higher (χ^2 =101.804, df 2; p<0.001) prevalence of LEAD (16.8%), than those having arsenic exposure only (Gr II) and those not having had such exposure (Gr III). The prevalence in Gr II and Gr III were 8.9% and 3.5% respectively (table-45).

The prevalence of LEAD was found to be significantly higher in members of Gr I than in members of Gr II and Gr III, both among males (χ^2=43.099, df 2; p<0.05) and females (χ^2=59.657, df 2; p<0.05). Among males the prevalence of LEAD was found to be 16.4%, 7.7% and 3.5% among members of Gr I, Gr II and Gr III respectively. And among females the prevalence of LEAD among members of Gr I, Gr II and Gr III were 17.1%, 9.7% and 3.5% respectively (table-45). In the current study though the prevalence of LEAD among males and females of Gr III was similar; the prevalence of LEAD was found to be higher amongst females than among males having excess arsenic exposure (Gr I & Gr II). This finding was similar to that of Tseng CH. et al (1996)[45] who reported LEAD (PVD) was more common amongst among females (15.7%) than males (7.2%) of blackfoot disease endemic villages of in southwestern Taiwan who had previous arsenic exposure and peripheral vascular disease but had ceased consumption of high-arsenic artesian well water for more than two decades.

Among males, those having arsenicosis and arsenic exposure (Gr I) and those having arsenic exposure only (Gr II) were 5.5 (95% CI 3.0-9.9) and 2.3 (95% CI 1.2-4.4) times more likely times to have LEAD, than those whose drinking water arsenic content was <0.05 mg/L (Gr III). Similarly among females, those having arsenicosis and arsenic exposure (Gr I) and those having arsenic exposure only (Gr II) were 5.6 (95% CI 3.5-9.2) and 2.9 (95% CI 1.8-4.9) times more likely times to have LEAD than those whose drinking water arsenic content was <0.05 mg/L (Gr III). Finally when adjusted for gender the risk of LEAD was still found to be higher among those whose drinking water arsenic content was >0.05 mg/L and had arsenicosis (OR 5.6; 95% CI 3.8-8.1), and those who had arsenic exposure (drinking water arsenic content >0.05 mg/L) but had not developed arsenicosis (OR 2.7; 95% CI 1.8-4.0) compared to those not having arsenicosis and whose drinking water arsenic content was < 0.05 mg/L (table 45). None the less, even if arsenicosis was ignored and following adjustment for gender, those having excess arsenic exposure (drinking water arsenic content > 0.05 mg/L) were about 4.1 (95% CI 2.8-5.8) times more likely ($p<0.001$) to have LEAD (table 47) compared to those having no such excess arsenic exposure (drinking water arsenic content <0.05 mg/L). Moreover compared to those having arsenic exposure but no arsenicosis (Gr II) individuals with arsenic exposure and arsenicosis (Gr I) were about 2.1 (95% CI 1.6-2.7) times more likely ($p<0.001$) to have LEAD even after adjustment for gender (table-46). These findings indicates that

With increasing age LEAD was found to be significantly ($p<0.001$) more prevalent in both males and females of all the study groups, and the prevalence were higher among participants of Gr I and Gr II than among participants of Gr III (table-32).

Age was found to be associated with an increasing prevalence of LEAD in all the study groups both among males [(Gr I: $\chi^2= 6.21$, df 2; $p=0.045$), (Gr II: $\chi^2= 20.484$, df 2; $p <0.001$) and (Gr III: $\chi^2= 21.221$, df 2; $p <0.001$) and females [(Gr I: $\chi^2= 6.473$, df 2; $p=0.039$), (Gr II: $\chi^2= 7.569$, df 2; $p <0.001$) and (Gr III: $\chi^2= 21.824$, df 2; $p <0.001$)]. Such association between age and an increasing prevalence of PVD in both genders was also observed by Tseng CH et al (1996)[45]. Moreover in all of the 3 study groups women had a higher prevalence of LEAD than men in almost all age groups. Tseng CH et al

(1996)[45] also reported similar observation among individuals residing in arsenic endemic villages of Taiwan.

When adjusted for age and gender the prevalence of LEAD (table-48) was still found to be higher in Gr I (16.8%) than that among participants of Gr II (9.2%) or Gr III (3.3%). When controlled for age and gender LEAD was about 5.9 (4.1-8.7) times more likely amongst those whose drinking water arsenic content was less than 0.05 mg/L and who had arsenicosis, and about 2.9 (1.9-4.3) times more likely to occur amongst those who have arsenic exposure (drinking water arsenic content >0.05 mg/L) but had not developed arsenicosis, compared to those whose drinking water arsenic content was less than 0.05 mg/L (χ^2= 162.351, df 5; p<0.001).

It was thus apparent from the current study that those having arsenic exposure (drinking water arsenic content >0.05 mg/L) were at higher risk of having LEAD than those having no such exposure (drinking water arsenic content <0.05 mg/L), and that those having arsenicosis were at greater risk. This observation is supported by the observation that peripheral vascular phenomena were more common among persons with abnormal skin pigmentation attributed to arsenic exposure, compared to that in persons with normal skin[40,41,241].

Smoking/Tobacco consumption

None of the female participants in any of the groups were either smokers, or had smoked in the past (table-34). The smoking status of the male participants (table-34 & 35) between the different study groups was not found to be significantly different (χ^2 =0.121, df 2; p=0.941). Smoking is a known risk factor for LEAD[1,2,5,7,21,23,252-255,297], therefore in estimating the risk of LEAD because of the group membership as designed in the current study arsenic the confounding effect smoking/tobacco use, as well as other possible confounders, were dealt with in the logistic regression procedure employing SPSSWin 11.5.

LEAD was found to be significantly more prevalent among participants of Gr I and Gr II than among participant of Gr III (table-60) both among ever smokers (χ^2= 14.659, df 2; p<0.001) and never smokers (χ^2= 89.544, df 2; p<0.001). Moreover when adjusted for smoking status LEAD was found to be 5.6 (3.8-8.1) and 2.7 (1.8-4.0) times more prevalent amongst members of Gr I and Gr II respectively compared to that in Gr III (χ^2=

106.328, df 3; p<0.001) (table-60). Again LEAD was found to be 4.0 (2.8-5.8) times more prevalent amongst those having excess arsenic exposure (table-62) compared to those having no such exposure (χ^2= 78.153, df 2; p<0.001) when adjusted for smoking status. Finally when adjusted for all tobacco consumption related variables (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco), age and gender together, LEAD was found to be about 5.7 and 2.7 times ore prevalent (χ^2= 181.535, df 9; p<0.001) among participants of Gr I and Gr II respectively compared to those belonging to Gr III (table-63).

Thus from the above findings it is evident that those having excess arsenic exposure (drinking water arsenic content >0.05mg/L) whether or not having arsenicosis were more likely to have LEAD and those having no such exposure, and those having arsenicosis in addition to excess arsenic exposure had a higher risk.

Obesity (BMI)

The BMI of the study participants (table no-37) in the different group were found not to be different (p>0.5). Among the participants 6.8% in Gr I, 8.7% in Gr II and 9.5% in Gr III were either overweight or obese. The groups were found not to be statistically different (χ^2=5.095, df 4; p>0.05) in terms of nutritional status (BMI).

When adjusted for overweight or obese LEAD was found to be about 5.5 (3.8-8.1) and 2.7 (1.8-4.0) times higher (χ^2 = 106.084, df 3; p<0.001) amongst members of Gr I and Gr II respectively in contrast to participants of Gr III (table-64 & 74).

DM

Diabetes is a known risk factor for LEAD[1,2,5,7,17,21,23,255,297]again exposure to arsenic through drinking water has been found to lead to increased prevalence of diabetes[65,150,239,339-341,403]. The prevalence of diabetes was 6.8%, 5.9% and 3.1% among participants of Gr I, Gr II and Gr III respectively, and the groups were found to be statistically different (χ^2 =14.925, df 2; p<0.05) in terms of diabetic status of the participants (table-38). LEAD was found to be more prevalent among participants of Gr I and Gr II than among participants of Gr III whether they were diabetic or non diabetic (table-74). When adjusted for DM status, age and gender (table-74), LEAD was found to be about 6.02 (4.11-8.81) and times 2.83 (1.89-4.25) more prevalent among

participants of Gr I and Gr II compared to that among participants of Gr III. When the dermatological manifestations of arsenicosis was ignored and participants having excess arsenic exposure was considered together it was found that when adjusted for diabetic status LEAD was found to be about 3.8 (2.1-6.8) times more common (χ^2=30.309, df 2; p<0.001) among those having excess arsenic exposure than those having no such exposure (table-74). LEAD was found to be about 4.0 (2.8-5.7) times more prevalent (χ^2=81.681, df 3; p<0.001) among members having excess arsenic exposure than among members having no such exposure when adjusted for diabetic status and gender.

Thus findings of this study also indicates that those having excess arsenic exposure (drinking water arsenic content >0.05mg/L) whether or not having arsenicosis were more likely to have LEAD and those having no such exposure, and those having arsenicosis in addition to excess arsenic exposure had a higher risk.

Hypertension (HTN)

HTN is a known risk factor for LEAD[1,2,5,7,17,21,23,247,250,297], again exposure to arsenic through drinking water has been found to lead increased prevalence of HTN[64,240] . The prevalence of HTN was 6.6%, 6.2% and 3.6% among participants of Gr I, Gr II and Gr III respectively. And the groups were found to be statistically different (χ^2 =52.453, df 4; p<0.001) in terms of HTN status of the participants (table-39). Adjusted for HTN status and gender, LEAD was found to be about 5.6 (3.8-8.1) and 2.7 (1.8-4.0) times more prevalent among participants of Gr I and Gr II respectively than among participants of Gr III (table-71). When adjusted for hypertension status and gender LEAD was found to be about 4.1 (2.8-5.8) times more prevalent among members of Gr I than among members of Gr II (table-72).

Again when adjusted for the differences in age, gender and HTN status between the study groups it was found that LEAD was about 6.1 (4.2-8.9) and 2.9 (1.9-4.3) times more prevalent among participants of Gr I and Gr II respectively than among participants of Gr III (table-74).

LEAD among the study groups adjusted for different study variables:

When adjusted for all socio-demographic variables (age, gender, household size, education level, occupation, housing type, annual household income, and household

possession of agricultural land, radio, bicycle, television and motorcycle); food habits related variables (staple food type, numbers of days of vegetable intake per week, number of days of fish/meat/egg intake per week, numbers of days of milk or milk products intake per week and cooking oil) together with age and gender; all tobacco consumption related variables (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco) along with age and gender, for all the different morbidity related variables (overweight/obesity, anemia, pedal edema, amputation, DM status and HTN) along with age and gender; and finally for all socio-demographic variables, all food habits related variables, all tobacco consumption related variables and all morbidity related together LEAD was found to be significantly more prevalent among members of Gr I and Gr II respectively than among members of Gr III. None the less LEAD was also found more prevalent among members of Gr I than among members of Gr II. And it was also evident even when presence or absence of arsenicosis was ignored LEAD was significantly more prevalent among those having excess arsenic exposure compared to those not having exposure to excess arsenic (table-75).

Thus the findings could thus be considered as indicative of that those having excess arsenic exposure through drinking water (≥0.05 mg/L) have enhanced chance of LEAD compared to those having no such exposure (<0.05 mg/L). And individuals with the dermatological manifestations of arsenicosis (bilateral palmoplantar keratosis and/or melanosis) were at higher risk of having LEAD arising out of such exposure.

5.6 ARSENIC IN DRINKING WATER & LEAD: DOSE EFFECT RELATIONSHIP

Increasing prevalence of LEAD was found with increasing cumulative arsenic exposure (χ^2 = 118.683, df 3; p<0.001). In the cumulative arsenic exposure category the prevalence of LEAD was found to be 2.0% and a prevalence of 17.6% was found in the highest cumulative arsenic exposure category of ≥10 mg-y/L (table-76). Increasing prevalence of LEAD with increasing cumulative arsenic exposure was observed both among males (p<0.001) and females (p<0.001).

Tseng CM et al (1996)[45] in a cross-sectional study on 582 adults (263 men and 319 women) aged 52.6±10.6 years having had previous arsenic exposure and peripheral

vascular disease but had ceased consumption of high-arsenic artesian well water for more than two decades in blackfoot disease endemic villages in Taiwan demonstrated a dose-response relation between the prevalence of peripheral vascular disease (ABI ≤0.90) and the long-term arsenic exposure. The ORs (95% confidence intervals) after adjustment for age, sex, body mass index, cigarette smoking, serum cholesterol and triglyceride levels, diabetes mellitus and hypertension were 2.77 (0.84-9.14) and 4.28 (1.26-14.54) for those who had cumulative arsenic exposure of 0.1- 19.9 and ≥ 20.0 mg-yr/L, respectively, compared with those who were not exposed. In the current study, higher risk of LEAD was observed with increasing cumulative arsenic exposure after adjustment for age, gender, body mass index, tobacco consumption (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco), obesity/overweight (based on BMI), diabetes mellitus and hypertension were found to be 2.808 (1.392- 5.664), 3.711 (2.104-6.548) and 4.889 (2.754-8.679) for those who had cumulative arsenic exposure of 1-4 mg-y/L, 5-9 mg-y/L and ≥10 mg-y/L respectively, compared with those who were not exposed (table-77).

Following adjustments for all socio-demographic variables (age; gender; educational status; occupation; family ownership of agricultural land, radio television, bicycle, motorcycle; household size housing type; and annual household income); food habit variables (staple food type, numbers of days of vegetable intake per week, number of days of fish/meat/egg intake per week, numbers of days of milk or milk products intake per week and cooking oil) along with age and gender; tobacco consumption (smoking status, years of smoking, habit of chewing tobacco and years of habit of chewing tobacco) along with age and gender; all morbidity related variables (overweight or obesity anaemia, pedal oedema, amputation, hypertension, diabetes, and skin manifestation-melanosis, keratosis) along with age and gender; all study variables except skin manifestation; and finally all study variables, LEAD was found to be more prevalent with (table-77) increasing cumulative arsenic exposure ($p<0.05$) in all of the scenarios considered for adjustments. Moreover the risk of LEAD was found to be higher if arsenic exposed individuals had the skin manifestations of arsenicosis, indicating that they were more sensitive to the effects of arsenic.

5.7 VALIDITY ASPECTS OF THE STUDY

Selection bias could have occurred if the arsenic-exposed subjects suffering LEAD or having traditional risk factors for LEAD were somehow more easily recruited to the study. However as the selection process was the same for all of the groups, selection bias if any would have probably affected all of the three study groups in a similar fashion and thus is not much expected to have influence the findings of the study.

Because of the lack of any sort of national or local registration systems and as the majority of the subjects being poor and illiterate some of the information elicited through interview for the purpose of the study could have been to some aspect affected; but as the groups were similar in terms of age, income and educational status the information for the all the groups were most likely to have been affected in a similar fashion and thus could have had little or no affect on the findings of the study. As the groups were found to be similar in terms of socio-demographic variables and as all participants were from rural areas the lifestyle were not expected to be substantially divergent.

In any epidemiological study, the possibility of uncontrolled confounding factor(s), which itself is a risk factor for the disease or is closely connected with a true risk factor is usually a matter of much concern, and controversy. Confounding occurs when the groups differ with regard to some determinant of the disease other than the exposure; that is there is a relationship between the confounding factor, the exposure under consideration and the disease in focus. Confounders are usually controlled by restriction and matching during participant recruitment or by stratification and adjustments during analysis or through use of multivariate analysis. In this study control for confounding and/or effect modification was undertaken by use of matching, stratification and or multivariate analysis. When studying LEAD it is plausible that there will be confounding with some known risk factors, namely age, sex, smoking, dietary habits, obesity (BMI), smoking (tobacco consumption), lipid abnormalities, diabetes mellitus and hypertension. The issues of confounding in regards to age, sex, smoking, dietary habits, obesity (BMI), smoking (tobacco consumption), diabetes mellitus and hypertension had been addressed in this study. Diabetes mellitus and hypertension though are individually risk factors for LEAD. Exposure to arsenic also been found to be associated with a higher risk of developing diabetes[65,339,341,403-405] and hypertension[64,240], both of which in turn can

accelerate the atherogenicity of arsenic and clinical development of obstructive vascular diseases. As for lipid abnormalities, Lee FN et al (1983)[280] in a study involving 49 hospitalized full blown BFD cases without diabetes, hypertension and other systemic diseases and 36 normal controls had reported that mean values of the lipid measurements concentrations (serum cholesterol, triglyceride, and HDL cholesterol) in BFD patients and control group to be were within the normal range, in another cross-sectional study[50] carried out a to examine whether lipid abnormalities contributed to the endemic peripheral vascular disease (PVD) in villages where arseniasis was hyperendemic in Taiwan it had been revealed that PVD in arsenicosis hyperendemic villages correlated with ingested inorganic arsenic and not with the lipid profiles. Based on these facts and because of feasibility and resource constraints, blood cholesterol or lipid profile was not included as a study variable. Individual with high blood cholesterol and or lipid abnormalities in the population are least likely to affect the findings of the study as they had equal chance of being recruited in all the three groups. It is not known whether other contaminants (eg, other trace elements, such as sodium, magnesium, manganese, iron, lead; and humic acid) could have been present in the water together with arsenic in the study areas. Moreover it is not clear whether such elements would have any stronger influence on atherogenecity, blood pressure or LEAD.

It is also worth mentioning that the design of this study was cross-sectional and the prevalence rather than incidence of LEAD had been used to assess the association between ingested inorganic arsenic and LEAD. As LEAD is associated with long-term arsenic exposure and as because those who develop LEAD are more likely to suffer from a poor survival[53], the odds ratios based on such prevalence data are more likely to have been underestimated. On the basis of such a conservative estimation, a significantly higher risk of LEAD was still found to be associated with long-term exposure to arsenic. This association remained significant after adjustment for conventional risk factors of LEAD.

Validity of Exposure Assessments

Arsenic, both in organic or inorganic forms occur in the soil, air, water, food and some medications[240,392,394]. Chronic exposures to arsenic from drinking water, coal burning, industrial sources or food are known to occur. In Bangladesh the exposure to arsenic

stems to the use ground water mainly tapped through hand operated tubewells and possibly crops and vegetables harvested in areas using this contaminated water for the purpose of irrigation. And all other routes of intakes of arsenic (intranasal and dermal) if any, are considered to be of minor importance in comparison to the oral route.

It has been revealed that some amount of arsenic occurs in crops, like uncooked rice, wheat, corn and vegetables harvested in the contaminated area and that significant intake of arsenic from foods cooked using contaminated water or foodstuffs made from livestock consuming contaminated drinking water can occur. As this research had focused on LEAD following oral exposure to arsenic, to define exposure accurately all consumed arsenic should have been considered. Estimation of arsenic in foodstuffs is quite cumbersome and difficult, and most studies reported from arsenic endemic areas poisoning had only considered inorganic form of arsenic in the drinking water in exposure assessments. In this study only the arsenic exposure through drinking water has been taken into consideration.

Quantification and retrospective assessments of exposures are always problematic, and this study was no exception in that respect. It would have been desirable to have directly measured individual exposure data which was dependent on the volume of water intake which is expected to vary by age, gender and occupation. Assessment of past individual exposure(s) to arsenic had some limitations. No written record of tubewells used in the past as drinking water sources and their arsenic content was available, and many of these sources used by study participants in the past had become non-existent and thus it was impossible to obtain past water samples. In such instances, water samples from three (3) existing tubewells closest to the site where the non-existent water source was located had been collected and the average arsenic concentration of water samples collected from these sources been used as the proxy for arsenic exposure in the past.

In situations as in the case of the present study, where drinking water level of arsenic had large variations or where there had been a long period of little arsenic exposure years, and in cases where adverse health effects appear after a long period of exposure as in the case of arsenic, the index of cumulative arsenic exposure could possibly most suitable in evaluation of dose–response relationships. In the approach the two parameters concerning the exposure, namely concentration and duration had been taken into consideration.

In Bangladesh waters from tubewells, even within the same village show a wide variation in arsenic concentration, so it is necessary to determine individual arsenic exposure by both duration and location of water consumption together with the measurement of arsenic concentration of all well waters that are involved. Despite the fact that arsenic concentration in a tubewell might not have the same as it was at the point of time when the study was carried out, for the purpose of this study it was assumed that the arsenic concentration in the tubewell had been fairly constant since the inception of the tubewell. Thus, the cumulative exposure estimates in is uncertain. Any use of bottled water would have diluted the effect and led to an underestimation of the associations found. Despite these uncertainities, it is reasonable to believe that the available water measurements were reliable enough to create the broad exposure categories used in the analysis of dose-response relationships. It is also possible that there was some information bias in the history of well water consumption, because the data were obtained from interviews. This type of bias was however a lesser concern, because neither those having LEAD nor those who were healthy were aware of which disease that was being studied. Furthermore, the investigators did not know the arsenic content of the drinking water when performing the household surveys.

Though no analysis of biological samples (e.g. hair, nail or urine), had been carried out in this to substantiate arsenic exposure in body, a major strength of this study was that it was the first large population-based study in Bangladesh with individual arsenic exposure data, which can provide critical information to characterize the exposure-response relationship with LEAD in reference to arsenic in drinking water both in presence and in absence of arsenic induced dermatological lesions.

Consistency of the Evidence

It is likely that there is a causal effect when an association of interest is repeatedly observed in separate investigations of different populations in different settings at different points in time. Considering arsenic exposure and LEAD (PAD/PVD), a causal connection several studies[45,268,275-278,400,401] on the association between long-term arsenic exposure and PVD risk. An increased prevalence of peripheral vascular disease has also been documented among inhabitants with long-term exposure to drinking water contaminated with high level of arsenic in Chile and Mexico[37,41]. A vasospastic disorder

of peripheral arteries, Raynaud's disease, has been reported to be associated with long-term environmental exposure to arsenic (Lagerkvist et al., 1988). Historically, ingested inorganic arsenic has been documented[43] to cause blackfoot disease, a unique endemic peripheral vascular disease in southwestern Taiwan. Tseng (1977)[43] reported the first dose–response relationship between ingested arsenic and prevalence of blackfoot disease. A 30-year follow-up study had shown that 68% of the 1300 clinical patients of blackfoot disease underwent spontaneous or operative amputation, and the re-amputation rate was 23.3%[49]. Switching to a surface water supply system in the arsenic-exposure area of southwestern Taiwan has resulted in a gradual decline of blackfoot disease incidence. In a case–control study on multiple risk factors for blackfoot disease, a dose–response relationship was found between the duration of consuming high arsenic artesian well water and the risk of blackfoot disease[268]. In a cross-sectional study was conducted to detect the dose–response relationship between PVD and chronic arsenic poisoning among residents in the arseniasis-endemic areas in Taiwan[45], using Doppler ultrasound measurements of systolic pressures to established the diagnosis of the PVD based on the ankle–brachial index <0.9 on either side (a more objective and sensitive tool to identify PVD), demonstrated a dose–response relationship between PVD prevalence and cumulative arsenic exposure after adjustment for age, gender, body mass index, cigarette smoking, and serum levels of total cholesterol and triglycerides. In another case–control study on arsenic in arterial tissues and risk of blackfoot disease, Wang et al., 2001[278] demonstrated that Blackfoot disease patients had higher arsenic contents in arterial tissues than unaffected controls. Yang CY[407] in the analysis of standardized mortality ratio of PVD among residents in the arseniasis-endemic areas in Taiwan from 1971 to 2003, demonstrated a gradual decline in PVD mortality after cessation of consumption of high-arsenic artesian well water.

The results of this study also indicates a strong dose-response relationship between arsenic in drinking water and LEAD in the population studied in Bangladesh, and is consistent with the findings of studies performed in Taiwan[45,47, 49,51,269,279].

The study was conducted in rural areas in Bangladesh, where the population lived under conditions of poverty and poor nutrition, which might have entailed increased sensitivity to arsenic toxicity. Studies have demonstrated that nutritional deficiencies can result in

impaired arsenic metabolism, methionine and vitamin C reduce arsenic toxicity, whereas vitamin A increases sensitivity to the element[276,370,407,409-425]. It has been demonstrated by a study in the BFD hyperendemic area in Taiwan[279] that individuals with a higher arsenic exposure and a lower capacity to methylate inorganic arsenic to DMA^V have a higher risk of developing PVD.

There is extensive malnutrition in Bangladesh, with very low protein and energy intake, which is compounded by a low level of vitamin A and iron in the diet[426-436]. It is possible that these nutritional deficiencies contribute to the enhanced susceptibility to arsenic toxicity and thus higher incidence chronic health effects arsenic exposure including LEAD.

Causal Inference

The most informative studies regarding effects of chronic arsenic exposure, especially LEAD, had been performed in Taiwan. In this Bangladeshi study LEAD also, appeared to be strongly associated with arsenic in drinking water. Nevertheless, concomitant exposure to various other elements in the water (e.g., manganese, iron, mercury, chromium, and lead) may also be of etiological importance, although at present there is no definite evidence to support that possibility. Humic substances have been mentioned as a cause of blackfoot disease (discussed above), and, hypothetically, they may cause diabetes mellitus. However, that can be refuted by the fact that no dose-related association been found between arsenic and humic substances in Taiwan. Besides if humic acid had any role in inducing LEAD participants in all of the groups this study would have been most likely similarly affected and hence could have least likely influenced the findings of the study. Therefore, arsenic may be the relevant element in drinking water that is primarily responsible for the increased prevalence of LEAD in Bangladesh.

The present study involved a relatively large number of subjects who were highly exposed as well, and we discerned a strong association between arsenic and LEAD including significant dose-effect relationships. Confounding and bias were apparently not a substantial problem, nor were they so strong as to hamper the validity of the observations made. The results of this investigation are probably reliable, because a fairly

large population was examined and data on individual exposure were of reasonably good quality.

5.8 IMPLICATIONS OF STUDY FINDINGS

Experiences and studies have documented [32,33,35-38,41--50,241,266-269,273,274,278,279] that long-term arsenic exposure was a dominant and independent risk factor for cardiovascular diseases including LEAD. Findings of this study, indicates existence of such an association among arsenic exposed population in Bangladesh, and also reinforces the earlier reports of occurrence gangrene in individuals with arsenicosis[53,54,57,58,61,68]. LEAD is considered a marker of atherosclerotic disease throughout the body[1-3,6,7,11,13,24,26]. An increased prevalence of peripheral vascular disease as well as ischemic heart disease has also been documented among inhabitants with long-term exposure to drinking water contaminated with high level of arsenic in Taiwan, Chile and Mexico[32,33,38-40,43-47,239,241,269-272,275,277,370,399-402,407]. The arsenic induced cardiovascular effects could be irreversible and/or persistent[45,399,402,437]. Besides the morbidity associated with progression of LEAD, patients of LEAD whether symptomatic or asymptomatic has been found to have a profound increased risk of cardiovascular ischemic events and mortality compared to the general population[1,2,5-8,10,11,13,15,19-25,27-31]. Therefore the arsenic exposed population in Bangladesh is not only at higher risk of morbidity associated with progression of LEAD but is also in increased risk of cardiovascular ischemic events and mortality. This study thus signals an emerging epidemic of impending of disability, cardiovascular ischemic events and mortality in the arsenic exposed population of Bangladesh if exposure remains unabated.

This study has demonstrated that population exposed to excess through drinking water (>0.05mg/L) whether or not having arsenical skin lesions (melanosis, keratosis, melanosis and keratosis) had a higher risk of having LEAD than those having no such exposure (drinking water arsenic ≥0.05mg/L). Those with arsenicosis were found to have a much higher risk than those not having such skin lesions, compared to those not having excess arsenic exposure (drinking water arsenic <0.05mg/L). None the less an increase in proportion of LEAD with increase in concentration of arsenic in drinking water was observed in this study. Similar observations have been made by Majumdar et al (2009)[438], in respect to systemic manifestations like chronic lung disease, chronic diarrhoea,

hepatomegaly, etc, they went further to comment that, it is worthwhile to include people with systemic manifestations with evidence of arsenic exposure as suspected cases of arsenicosis, and that they need to be followed up for a prolonged period for detection of arsenical skin lesion and cancer on a later date. Thus any surveillance and intervention programmes designed to mitigate effects of arsenic exposure should include LEAD as a health effect, and should target not only individuals with arsenicosis but all individuals having excess arsenic exposure even if they did not have arsenical skin lesions.

Implementation of surface-water supply system in the arsenic-exposure area of southwestern Taiwan has resulted in declining incidence of blackfoot disease and IHD mortality[437]. For the mitigation of arsenic-related environmental catastrophe, the supply of drinking water with low level of arsenic is extremely important. Although the discontinuation of the use of high arsenic-containing drinking water remains to be the most efficient way of prevention of arsenic-related cardiovascular disease, the improvement of the nutritional status (e.g., supplementation with antioxidants) and the facilitation of the individual capability to methylate inorganic arsenic may have public health significance on the prevention of the development of arsenic-related diseases in the already-exposed subjects.

CHAPTER VI

CONCLUSIONS & RECOMMENDATIONS

6.1 CONCLUSIONS

Based on the current cross sectional comparative study the following conclusions can be drawn:

1. In Bangladesh lower extremity arterial disease (LEAD) was prevalent amongst the populations as represented by the different study groups.
2. The prevalence and risk of lower extremity arterial disease (LEAD) was higher amongst the population in Bangladesh whose drinking water source(s) contains arsenic in excess of 0.05 mg/L. than those whose drinking water source(s) does not contain excess arsenic (<0.05mg/L).
3. Among those whose drinking water source(s) contains arsenic in excess of 0.05 mg/L, the prevalence and risk of lower extremity arterial disease was higher if they had developed the signs of arsenicosis (melanosis ± keratosis).
4. A dose response relationship between cumulative arsenic exposure and lower extremity arterial disease (LEAD) exists in the population in Bangladesh as represented by the study groups.

6.2 RECOMMENDATIONS

Based on the findings and observations made in this study the following recommendations have been drawn:

1. To minimize the risk of lower extremity arterial disease (LEAD) and it's related morbidities and mortalities, steps for urgent cessation of use of arsenic contaminated water for drinking needs to be taken.
2. Individuals in arsenic endemic areas having lower extremity arterial disease, both symptomatic and asymptomatic need to be identified objectively and kept under continued yearly follow up.
3. Individuals with significant LEAD in arsenic endemic areas should be brought under appropriate medical intervention.
4. Programmes aiming for reduction of modifiable atherosclerotic risk factors (obesity/overweight, smoking, arsenic consumption, diabetes, hypertension, etc) needs to be taken targeting population living in arsenic endemic areas and should include all exposed individuals not only those having the dermatological signs of arsenicosis.
5. Surveillance programmes for health effects of arsenic exposure particularly cancers, Diabetes mellitus, hypertension, LEAD, CAD, CVD and other health effects attributable to excess arsenic exposure should include all exposed individuals, not only those having the dermatological signs of arsenicosis.
6. A cohort study with arsenic safe water intervention aiming to stop or reduce arsenic exposure may be under taken to explore the role of such intervention in reduction of lower extremity arterial disease in Bangladesh.
7. A cohort study if under taken to establish casual relationship between arsenic exposure and lower extremity arterial disease in Bangladesh will strengthen the findings of this study and further add to the current state of knowledge in respect to lower extremity arterial disease and other atherosclerotic diseases arising out of arsenic exposure through drinking water.

CHAPTER VII

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7.0 REFERENCES

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ANNEXURES

ANNEX-I

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh

অংশগ্রহণকারী নির্বাচনী ফর্ম

আই ডি নং | | | | | |

আর্সেনিকোসিস (arsenicosis) গ্রুপ (গ্রুপ -১)

অংশগ্রহণকারীর বর্তমান বাসস্থান

গ্রাম: _____ উপজেলা: _____ জেলা: _____

অংশগ্রহণকারীর জন্মস্থান

আপনি যেখানে জন্মগ্রহন করেছেন সেই গ্রাম, উপজেলা (থানা) ও জেলার নাম বলুন

গ্রাম: _____ উপজেলা: _____ জেলা: _____

৩	এই থানা/উপজেলায় আপনি কত বৎসর যাবৎ বসবাস করছেন	__/__/	অন্যান্য=০ জন্ম থেকে=১	☐ **উত্তর "০" হলে ইনটারভিউ বন্ধ করুন**
৪	এই গ্রামে আপনি কত বৎসর যাবৎ বসবাস করছেন	__/__/	১০ বৎসরের কম সময় ধরে=০ ১০ বৎসর বা তার চেয়ে বেশী সময় ধরে=১ জন্ম থেকে=২	☐ **উত্তর "০" হলে ইনটারভিউ বন্ধ করুন**
৫	বর্তমানে আপনি কোন উৎস থেকে সংগ্রহ করা পানি খেয়ে/পান করে থাকেন		পুকুর/নদী/কুয়া/হাঁন্দরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২	☐
৬	আপনার খাবার পানি যে সকল টিউবওয়েল / ডিপ টিউবওয়েল থেকে সংগ্রহ করা হতো সেগুলোর মধ্যে কোনটিতে অতিরিক্ত আর্সেনিক ধরা পড়েছে কি		না = ০ হা= ১	☐
৭	আপনার খাবার পানি যে সকল টিউবওয়েল / ডিপ টিউবওয়েল থেকে সংগ্রহ করা হতো সেগুলোর মধ্যে কোনটিতে অতিরিক্ত আর্সেনিক ধরা পরার কারণে লাল রং করা হয়েছে কি		না = ০ হা= ১	☐
	৬ নং প্রশ্নের উত্তর "০" হলে, উত্তরের যথার্থতা সম্পর্কে নিশ্চিত হয়ে ইনটারভিউ বন্ধ করুন			
৮	আপনি কি বর্তমানে মারাত্মক বা অত্যধিক রক্তশূন্যতায় ভূগছেন		হা =-০ না=১ (উত্তর যাচাইপূর্বক রেকর্ড করুন)	☐ **উত্তর "০" হলে ইনটারভিউ বন্ধ করুন**

শুরু থেকে শেষ পর্যন্ত ইনটারভিউ সফলভাবে সম্পন্ন করা সম্ভব হয়েছে কি? না ☐ হা ☐

যাদের ক্ষেত্রে শুরু থেকে শেষ পর্যন্ত ইনটারভিউ সফলভাবে সম্পন্ন করা সম্ভব হয়েছে তারাই কেবল আর্সেনিকোসিস (arsenicosis) গ্রুপে অংশগ্রহণকারী হিসাবে নির্বাচিত হবেন

আর্সেনিকোসিস (arsenicosis) গ্রুপে অংশগ্রহণকারী হিসাবে

নির্বাচিত ☐ **নির্বাচিত না** ☐

ইনটারভিউকারীর স্বাক্ষর _____

তারিখ _____

আর্সেনিকোসিস (arsenicosis) গ্রুপে অংশগ্রহণকারী হিসাবে নির্বাচিত হলে অংশগ্রহণকারীর সম্মতি পত্র প্রয়োগ করুন

ANNEX- II

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh

অংশগ্রহণকারী নির্বাচনী ফর্ম

আই ডি নং | | | | | |

আর্সেনিকোসিস ছাড়া আর্সেনিক একস্পোজার গ্রুপ **গ্রুপ -২**

Arsenic exposure group without arsenicosis)

অংশগ্রহণকারীর বর্তমান বাসস্থান

গ্রাম: _____ উপজেলা: _____ জেলা: _____

অংশগ্রহণকারীর জন্মস্থান

আপনি যেখানে জন্মগ্রহন করেছেন সেই গ্রাম, উপজেলা (থানা) ও জেলার নাম বলুন

গ্রাম: _____ উপজেলা: _____ জেলা: _____

৩	এই থানা/উপজেলায় আপনি কত বৎসর যাবৎ বসবাস করছেন	__/__/	অন্যান্য=০ জন্ম থেকে=১	উত্তর "০" হলে ইনটারভিউ বন্ধ করুন
৪	এই গ্রামে আপনি কত বৎসর যাবৎ বসবাস করছেন	__/__/	১০ বৎসরের কম সময় ধরে=০ ১০ বৎসর বা তার চেয়ে বেশী সময় ধরে=১ জন্ম থেকে = ২	উত্তর "০" হলে ইনটারভিউ বন্ধ করুন
৫	বর্তমানে আপনি কোন উৎস থেকে সংগ্রহ করা পানি খেয়ে/পান করে থাকেন		পুকুর/নদী/কুয়া/ইন্দিরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২	
৬	আপনার খাবার পানি যে সকল টিউবওয়েল / ডিপ টিউবওয়েল থেকে সংগ্রহ করা হতো সেগুলোর মধ্যে কোনটিতে অতিরিক্ত আর্সেনিক ধরা পড়েছে কি		না = ০ হা= ১	
৭	আপনার খাবার পানি যে সকল টিউবওয়েল / ডিপ টিউবওয়েল থেকে সংগ্রহ করা হতো সেগুলোর মধ্যে কোনটিতে অতিরিক্ত আর্সেনিক ধরা পরার কারণে লাল রং করা হয়েছে কি		না = ০ হা= ১	
৬ নং প্রশ্নের উত্তর "০" হলে, উত্তরের যথার্থতা সম্পর্কে নিশ্চিত হয়ে ইনটারভিউ বন্ধ করুন				
৮	আপনি কি বর্তমানে মারাত্মক বা অতিরিক্ত রক্তশূণ্যতায় ভুগছেন		হা=-০ না=১ (উত্তর যাচাইপূর্বক রেকর্ড করুন)	উত্তর "০" হলে ইনটারভিউ বন্ধ করুন

শুরু থেকে শেষ পর্যন্ত ইনটারভিউ সফলভাবে সম্পন্ন করা সম্ভব হয়েছে কি? না ☐ **হা** ☐

যাদের ক্ষেত্রে শুরু থেকে শেষ পর্যন্ত ইনটারভিউ সফলভাবে সম্পন্ন করা সম্ভব হয়েছে তারাই কেবল আর্সেনিকোসিস ছাড়া আর্সেনিক একস্পোজার গ্রুপে **(Arsenic exposure group without arsenicosis)** অংশগ্রহণকারী হিসাবে নির্বাচিত হবেন

আর্সেনিক একস্পোজার গ্রুপে **(Arsenic exposure group without arsenicosis)**

নির্বাচিত ☐ নির্বাচিত না ☐

ইনটারভিউকারীর স্বাক্ষর _____

তারিখ _____

আর্সেনিক একস্পোজার গ্রুপে **(Arsenic exposure group without arsenicosis)** নির্বাচিত হিসাবে নির্বাচিত হলে অংশগ্রহণকারীর সম্মতি পত্র প্রয়োগ করুন ।

ANNEX-III

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh

অংশগ্রহণকারী নির্বাচনী ফর্ম

আই ডি নং | | | | | |

রেফারেন্স গ্রুপ (গ্রুপ -৩) [Reference Group]

অংশগ্রহণকারীর বর্তমান বাসস্থান

গ্রাম: _____ উপজেলা: _____ *জেলা: _____

অংশগ্রহণকারীর জন্মস্থান

আপনি যেখানে জন্মগ্রহন করেছেন সেই গ্রাম, উপজেলা (থানা) ও জেলার নাম বলুন

গ্রাম: _____ উপজেলা: _____ জেলা: _____

৩	এই থানা/উপজেলায় আপনি কত বৎসর যাবৎ বসবাস করছেন	__/__/	অন্যান্য=০ জন্ম থেকে=১	☐ উত্তর "০" হলে ইনটারভিউ বন্ধ করুন
৪	এই গ্রামে আপনি কত বৎসর যাবৎ বসবাস করছেন	__/__/	১০ বৎসরের কম সময় ধরে = ০ ১০ বৎসর বা তার চেয়ে বেশী সময় ধরে = ১ জন্ম থেকে = ২	☐ উত্তর "০" হলে ইনটারভিউ বন্ধ করুন
৫	বর্তমানে আপনি কোন উৎস থেকে সংগ্রহ করা পানি খেয়ে/পান করে থাকেন		পুকুর/নদী/কুয়া/হাঁদরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২	☐
৬	আপনার খাবার পানি যে সকল টিউবওয়েল / ডিপ টিউবওয়েল থেকে সংগ্রহ করা হতো সেগুলোর মধ্যে কোনটিতে অতিরিক্ত আর্সেনিক ধরা পড়েছে কি		না = ০ হা = ১	☐
৭	আপনার খাবার পানি যে সকল টিউবওয়েল / ডিপ টিউবওয়েল থেকে সংগ্রহ করা হতো সেগুলোর মধ্যে কোনটিতে অতিরিক্ত আর্সেনিক ধরা পরার কারণে লাল রং করা হয়েছে কি		না = ০ হা = ১	☐
৬ নং প্রশ্নের উত্তর "১" হলে, উত্তরের যথার্থতা সম্পর্কে নিশ্চিত হয়ে ইনটারভিউ বন্ধ করুন				
৮	আপনি কি বর্তমানে মারাত্মক বা অতিক্তি রক্তশূণ্যতায় ভূগছেন		হা=-০ না=১ (উত্তর যাচাইপূর্বক রেকর্ড করুন) উত্তর "০" হলে ইনটারভিউ বন্ধ করুন	☐

শুরু থেকে শেষ পর্যন্ত ইনটারভিউ সফলভাবে সম্পন্ন করা সম্ভব হয়েছে কি? না ☐ হা ☐

যাদের ক্ষেত্রে শুরু থেকে শেষ পর্যন্ত ইনটারভিউ সফলভাবে সম্পন্ন করা সম্ভব হয়েছে তারাই কেবল রেফারেন্স গ্রুপে **[Reference Group]** অংশগ্রহণকারী হিসাবে নির্বাচিত হবেন

রেফারেন্স গ্রুপে [Reference Group] অংশগ্রহণকারী হিসাবে

নির্বাচিত ☐ নির্বাচিত না ☐

ইনটারভিউকারীর স্বাক্ষর _____

তারিখ _____

রেফারেন্স গ্রুপে **[Reference Group]** নির্বাচিত অংশগ্রহণকারীর হিসাবে নির্বাচিত হলে অংশগ্রহণকারীর সম্মতি পত্র প্রয়োগ করুন ।

ANNEX-IV

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh

অংশগ্রহণকারীর জন্য তথ্যাবলি

আপনি খাবার জন্য যে পানি ব্যবহার করেন তাতে বিভিন্ন মাত্রায় আর্সেনিক থাকতে পারে । আর্সেনিক যুক্ত পানি পান করার কারনে শরীরের চামড়ায় কিছু লক্ষণ দেখা দেয়া ছাড়াও ডাইয়াবিটিস বা বহুমূত্র রোগ, উচ্চ রক্তচাপ বা ব্লাড প্রেসার রোগ, পায়ের শিরার (রক্ত নালীর) রোগ দেখা দিতে পারে । আমরা নিপসম থেকে এই গবেষনার মাধ্যমে আর্সেনিক যুক্ত পানি পান করার কারনে পায়ের শিরার (রক্ত নালীর) রোগের প্রাদূরভাব নির্ণয়ের জন্য একটি গবেষণা হাতে নিয়েছি । আপনিও এই গবেষনায় অংশগ্রহণকারী হতে পারেন ।

তবে, যেহেতু গবেষনায় অন্তর্ভূক্তির জন্য কিছু বৈশিষ্ট্যমালা আছে, সেহেতু যারা ঐ বৈশিষ্ট্যমালার আলোকে অন্তর্ভূক্তির জন্য যোগ্য বলে বিবেচিত হবেন কেবল তাদেরকেই সম্মতি প্রদান সাপেক্ষে গবেষনায় অংশগ্রহনকারী হিসেবে অন্তর্ভূক্ত করা হবে। এই গবেষনায় অংশগ্রহনের কারনে আপনার কোন খরচ হবে না ।

আপনি যদি এই গবেষনায় অন্তর্ভূক্ত হন তবে আপনাকে আপনার বাড়ীঘর, আপনার কাজ, আপনার আয়, আপনার পানির উৎস, আপনার দৈনন্দিন খাবার ও আপনার স্বাস্থ্য সম্পর্কে কিছু প্রশ্ন করা হবে এবং আপনার স্বাস্থ্য পরীক্ষা করা হবে। আপনার স্বাস্থ্য পরীক্ষার জন্য এবং পায়ের শিরার (রক্ত নালীর) রোগের প্রাদূরর্ভাব নির্ণয়ের জন্য এবং একটি নির্দিষ্ট দিনে আপনাকে থানা স্বাস্থ্য কেন্দ্রে /নির্ধারিত স্থানে, নির্দিষ্ট তারিখে ও সময়ে খালি পেটে (না খেয়ে) উপস্থিত হওয়ার জন্য অনুরোধ করা হবে । ডাইয়াবিটিস বা বহুমূত্র রোগ নির্ণয়ের জন্য আপনার নিকট থেকে দুই ফোটা রক্ত সংগ্রহ করা হবে । তা ছাড়াও আপনি যে সকল উৎস থেকে খাবার পানি সংগ্রহ করছেন এবং সংগ্রহ করতেন সে সকল উৎস থেকে পানির নমুনা সংগ্রহ করে আর্সেনিকের মাত্রা নির্ণয় করা হবে ।

আমরা আপনার ব্যক্তিগত তথ্যগুলি এবং সাক্ষাতকারের সব তথ্য সম্পুর্ণ গোপন রাখব । আমরা যখন গবেষনার কথা বলব বা লিখব তখন আমরা কখনও আপনাব নাম উল্লেখ করব না বা এমন কোন তথ্য প্রকাশ কবব না যাহাতে আপনাব পরিচিতি প্রকাশ পায় ।

আপনি যদি চান তবে আপনার রক্ত ও স্বাস্থ্য পরীক্ষার রিপোর্ট আপনাকে/ আপনার স্বাস্থ্য সেবা প্রদানকাবীকে দিতে পারি ।

আপনার যদি কোন ধরনের জিজ্ঞাসা থাকে তবে গবেষকগণের সাথে যোগাযোগ করুন :

ডাঃ মনজুরুল হক খান (০১৫২৩০৩৫৩২), সহকারী অধ্যাপক, পেশা ও পরিবেশ স্বাস্থ্য বিভাগ, নিপসম মহাখালী, ঢাকা - ১২১২ । অধ্যাপক (ডাঃ) শেখ আখতার আহমদ (৯৮৯৮৭৭১), বিভাগীয় প্রধান, পেশা ও পরিবেশ স্বাস্থ্য বিভাগ, নিপসম মহাখালী, ঢাকা - ১২১২ ।

গবেষনায় অন্তর্ভূক্তির জন্য বৈশিষ্ট্যমালার আলোকে যোগ্য বলে বিবেচিত হলে আপনি যদি গবেষনায় অন্তর্ভূক্ত হতে চান তবে অনুগ্রহ পূর্বক সম্মতি পত্রে আপনাব স্বাক্ষর / টিপসহি প্রদান করুন ।

ডাঃ মনজুরুল হক খান,
সহকারী অধ্যাপক,
পেশা ও পরিবেশ স্বাস্থ্য বিভাগ,
নিপসম মহাখালী, ঢাকা - ১২১২ ।

বিঃ দ্রঃ

সকল সম্ভাব্য অংশগ্রহণকারীকে অংশগ্রহণকারীর জন্য তথ্যাবলি স্পষ্ট ভাবে বর্ননার পর, অংশগ্রহণকারী নির্বাচনী ফর্ম- এর মাধ্যমে গবেষনায় অন্তর্ভূক্তির যোগ্যতা যাচাই করতে হবে ।

অংশগ্রহণকারীর সম্মতি পত্র

আই ডি নং | | | | | |

আমি

নাম: _____

বাবার নাম: _____

স্বামীর নাম: (প্রযোজ্য ক্ষেত্রে): _____

(২) বর্তমান বাসস্থান সম্পর্কিত

বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): _____

বাড়ীর নাম (যদি থাকে): _____

পাড়ার নাম (যদি থাকে): _____

গ্রাম: _____ ওয়ার্ড : _____ ইউনিয়ন: _____

মৌজা: _____ ** উপজেলা: _____ **জেলা: _____

(৩) জন্মস্থান সম্পর্কিত

আমার জন্মস্থান :

গ্রাম: _____ ওয়ার্ড : _____ ইউনিয়ন: _____

মৌজা: _____ **উপজেলা: _____ **জেলা: _____

এই গবেষনাব উদ্দেশ্য, এই গবেষনায় কি করা হবে, আমার কি করণীয় এবং আমার প্রতি গবেষকের/ গবেষকদের দায়িত্ব সম্পর্কে ভালভাবে জানার পর এই গবেষনায় অন্তর্ভূক্ত হবার জন্য স্বেচ্ছায় এই সম্মতি পত্রে নাম স্বাক্ষর / টিপসহি প্রদান করলাম ।

স্বাক্ষর/টিপসহি _____ তারিখ _____

আমার স্বাস্থ্য পরীক্ষা, ও পায়ের শিরার (রক্ত নালীর) পরীক্ষার রিপোর্ট (ফলাফল) আমার স্বাস্থ্য সেবা প্রদানকারী কর্তৃপক্ষের নিকট / আমার নিকট দিতে হবে ।

_____
প্রযোজ্য হলে
স্বাক্ষর/টিপসহি

ইনটাবভিউকারীর নাম: _____

ইনটারভিউকারীর স্বাক্ষর _____ তারিখ _____

১. আর্সেনিকোসিস (arsenicosis) গ্রুপে নির্বাচিত অংশগ্রহণকারীর জন্য Household ফর্ম পূরণ করুন ।

২. অংশগ্রহণকারীর স্বাক্ষরযুক্ত সম্মতি পত্র Household interview schedule(ফর্ম)-এর শেষে যুক্ত করুন ।

বিঃ দ্রঃ যে সমস্ত অংশগ্রহণকারী পড়তে ও স্বাক্ষর করতে অক্ষম তাঁদের ক্ষেত্রে স্পষ্ট ভাবে সম্মতি পত্র বর্ণনার পর টিপসহি সংগ্রহ করতে হবে ।

গবেষকের অংশ

ANNEX-VI

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh

HOUSEHOLD INTERVIEW SCHEDULE আইডি নং | | | | | |

নিপসমের পেশা ও পরিবেশ স্বাস্থ্য বিভাগ থেকে আর্সেনিক যুক্ত পানি পান করার কারনে পায়ের শিরার (রক্ত নালীর) রোগের প্রাদূর্ভাব নির্ণয়ের জন্য পরিকল্পিত এই গবেষনায় অংগ্রহনের সম্মতি প্রদানে আপনাকে অসংখ্য ধন্যবাদ ।

উপজেলা _____ জেলা _____

ইনটারভিউ গ্রহণের তারিখ | | | | | 2 | 0 | 0 | | (দিন/মাস/বৎসর)

ইনটারভিউ আরম্ভের সময়: _____ ইনটারভিউ শেষ করার সময়: _____

ইনটারভিউকারীর নাম: _____

ইনটারভিউকারীর স্বাক্ষর _____

প্রশ্নমালা যাচাইকারীর নাম ও স্বাক্ষর

নাম _____

স্বাক্ষর _____ তারিখ _____

ANNEX-VI

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh

HOUSEHOLD INTERVIEW SCHEDULE আইডি নং | | | | | |

বাসস্থানের ইতিহাস		
আপনি এই বাড়ীতে কত দিন ধরে বসবাস করছেন ?	জন্ম থেকে =০ অন্যান্য =১	
	কত বৎসর ধরে এই বাড়ীতে বসবাস করছেন?	
বর্তমানে যে বাড়ীতে বসবাস করছেন সেখানে জন্ম থেকে বসবাস না করে থাকলে এর পূর্বে যে সকল স্থানে বসবাস করেছেন তার পর্যায়ক্রমিক বিবরণ (অবস্থানের মেয়াদ সহ) ।	বাড়ীর নাম (যদি থাকে): বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা: বাড়ী প্রধানের নাম: বাড়ী প্রধানের সাথে সম্পর্ক এই বাড়ীতে কত দিন ধরে বসবাস করেছেন?	
	বাড়ীর নাম (যদি থাকে): বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা: বাড়ী প্রধানের নাম: বাড়ী প্রধানের সাথে সম্পর্ক এই বাড়ীতে কত দিন ধরে বসবাস করেছেন?	
	বাড়ীর নাম (যদি থাকে): বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা: বাড়ী প্রধানের নাম: বাড়ী প্রধানের সাথে সম্পর্ক এই বাড়ীতে কত দিন ধরে বসবাস করেছেন?	

ANNEX-VI

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh

HOUSEHOLD INTERVIEW SCHEDULE আইডি নং ☐☐☐☐☐

আর্থসামাজিক তথ্য (Sociodemographic information)			
১	বর্তমানে আপনার বয়স কত ? (পূর্ণ বৎসরে)		☐☐
২	লিঙ্গ	পুরুষ= ০ মহিলা= ১	☐
৩	আপনি কোন ধর্ম পালন করেন	ইসলাম =০ হিন্দু =১ খ্রীষ্টান =২ বৌদ্ধ =৩ অন্যান্য=৯	☐
৪	আপনি কি বিবাহিত	অবিবাহিত =০ বিবাহিত=১ বিধবা/ বিপত্নীক / তালাকপ্রাপ্ত =২	☐
৫	আপনার পরিবারের/খানার মোট সদস্য সংখ্যা কত?		☐☐
৬.০	আপনি কি কখনো স্কুলে গিয়েছেন	না=০ হা=১	☐
৬.১	আপনি সর্বোচ্চ কোন ক্লাশ পর্যন্ত পড়াশুনা করেছেন	[প্রযোজ্য না হলে = ০ ০]	☐☐
৬.২	স্কুলে যেয়ে থাকলে কোন পর্যন্ত পড়াশুনা করেছেন	কখনো স্কুলে যাই নাই = ০ প্রাইমারী = ১ মাধ্যমিক/ দাখিল = ২ উচ্চ মাধ্যমিক/ আলিম = ৩ বিশ্ববিদ্যালয় / ফাজিল/ কামিল =৪ প্রত্যাখ্যান=৯	☐
৭	আপনার পেশা কি	বেকার=০ গৃহবধু/গৃহিনী/বাড়ীর কাজ =১ ছাত্র/ ছাত্রী = ২ কৃষি কাজ =৩ রিক্সা চালক/ভ্যান চালক/মাঝি/দিন মজুর/জেলে =৪ ব্যবসা/দোকানদার/হকার/ ফেরীওয়ালা=৫ চাকুরী=৬ অবসর প্রাপ্ত/পেনশন প্রাপ্ত =৭ রং মিস্ত্রী / কাপড় রং করার কাজ =৮ অন্যান্য =৯	☐☐ উত্তর "৯" হলে নির্দিষ্ট পেশা নীচে লিপিবদ্ধ করুন _____
৮	কত দিন যাবৎ (বৎসর)আপনি এই পেশার সাথে জড়িত	[প্রযোজ্য না হলে = ০ ০]	☐☐
৯	আপনার পেশার কারনে গড়ে দৈনিক কত ঘন্টা আপনাকে বাড়ীর বাহিরে থাকতে হয়	[প্রযোজ্য না হলে = ০ ০]	☐☐
১০	আপনাদের কি পরিমাণ কৃষি জমি আছে? *১০০ শতাংশ =১ একর = ৩ বিঘা* *১০০০ শতাংশ =১০ একর = ৩০ বিঘা* ২০ কাঠা = *১ বিঘা*	জমির পরিমাণ _____ বিঘা কৃষি জমি নাই =০ ৩ বিঘা (১০০ শতাংশ) এর কম =১ ৩-২৫ বিঘা =২ ২৫ বিঘা এর বেশী =৩	☐

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আর্থসামাজিক তথ্য (Sociodemographic information)			
১১	বসত বাড়ীর ধরন (পর্যবেক্ষন করুন)	কাচা ভিটি+ বেড়ার দেয়াল+বেড়ার ছাদ =০ কাচা ভিটি+বেড়ার দেয়াল+টিনের ছাদ =১ কাচা ভিটি+ টিনের/মাটির দেয়াল+টিনের/টালির ছাদ =২ পাকা ভিটি+ টিনের দেয়াল+টিনের/টালির ছাদ =৩ পাকা ভিটি+ পাকা দেয়াল+টিনের/টালির ছাদ =৪ পাকা ভিটি+ পাকা দেয়াল+পাকা ছাদ =৫ অন্যান্য =৯	
১২	আপনার বাড়ীতে টেলিভিশন আছে	না=০ হা=১	
১৩	আপনার বাড়ীতে ডিভিও/ভি-ইন ওয়ান আছে	না=০ হা=১	
১৪	আপনার বাড়ীতে ফ্রিজ আছে	না=০ হা=১	
১৫	আপনার পারিবারের কোন সদস্যের সাইকেল আছে	না=০ হা=১	
১৬	আপনার পারিবারের কোন সদস্যের মটর সাইকেল আছে	না=০ হা=১	
১৭	আপনার পারিবারের কোন সদস্যের মটর গাড়ী আছে	না=০ হা=১	
১৮	আপনার পরিবারের কত জন উপার্যন বা আয় করেন		

১৯	পরিবারের উপার্যন বা আয়কারী সদস্যের নাম	পেশা থেকে আয় (বাৎসরিক)	কৃষি জমি থেকে আয় (বাৎসরিক)	অন্যান্য খাত থেকে আয় (গরু-ছাগল পালন, মৎস চাষ, হাস-মুরগী পালন, হস্ত শিল্প, ইত্যাদি)(বাৎসরিক)	মোট আয় (বাৎসরিক)
ক					
খ					
গ					
ঘ					
ঙ					
চ					
	সর্বমোট পারিবারক আয়				

২০	আপনার বৎসরিক ব্যক্তিগত আয় কত টাকা	

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খাদ্যাবাস

	খাদ্যাভ্যাস (Food habit- food consumption frequency)		
২১	প্রতিদিন আপনি প্রধান খাবার হিসাবে কি খেয়ে থাকেন ? (staple food)	ভাত =০ ভাত +ডাল=১ ভাত+আলু=২ ভাত+ডাল+আলু=৩ অন্যান্য=৪	
২২	আপনি সাধরনত সপ্তাহে কয় দিন শাক্‌সব্জি খেয়ে থাকেন ?	শাক্‌সব্জি মানে যে কোন শাক্‌, লাউ, কুমড়া, শিম, বরবটি, ঢেড়স, ডাটা, করল্লা, চিচিংগা, পেপে, ঝিংগা, মূলা, বাধা(পাতা) কফি, ফুল কফি, পটল, মুলামুা, টমেটো, লাজম, ইত্যাদি	
২৩	আপনি সাধরনত সপ্তাহে কয় দিন মাছ, মাংস বা ডিম খেয়ে থাকেন		
২৪	আপনি সাধরনত সপ্তাহে কয় দিন দুধ, লেবা বা দুধ থেকে তৈরা খাবার খেয়ে থাকেন ?		
২৫	আপনার বাড়াতে রান্নায় সাধরনত কোন তৈল ব্যবহার হয়ে থাকে	সয়াবিন তৈল = ০ সয়াবিন তৈল= ১ বাদাম তৈল = ২ পাম ওয়েল= ৩ নির্দিষ্ট কিছু না/ যা পাই= ৪ জানি না = ৯	

তামাক জাত দ্রব্য ব্যবহারের অভ্যাস

২৬.০ ধুমপান অভ্যাস (Smoking habit)			
২৬.১	ধুমপানের তথ্য আপনি কখনো ধুমপান করেছেন কি ?	বর্তমানে ধুমপান করি = ২ *বর্তমানে ধুমপান করি করি না পূর্বে করেছি* = ১ কখনো ধুমপান করি নাই = ০	
২৬.২	আপনি কত বৎসর যাবত ধুমপান করছেন / করেছেন ? [প্রযোজ্য না হলে = ০ ০]		
২৭.০ জর্দা বা সাদা পাতা খাওয়ার অভ্যাস			
২৭.১	আপনার জর্দা বা সাদা পাতা খাওয়ার অভ্যাস আছে কি ?	না=০ হা = ১	
২৭.২	আপনি কত বৎসর যাবত জর্দা বা সাদা পাতা খান ? [প্রযোজ্য না হলে = ০ ০]		

২৮.০	বহুমূত্র রোগ (Diabetes)		
২৮.১	আপনার কি ডাইয়াবিটস বা বহুমূত্র রোগ আছে ?	না=০ হা=১	
২৮.২	কত বৎসর যাবত আপনি ডাইয়াবিটস বা বহুমূত্র রোগে ভূগছেন ? [প্রযোজ্য না হলে = ০ ০]		
২৮.৩	ডাইয়াবিটস বা বহুমূত্র রোগের জন্য আপনি কি বর্তমানে কোন ঔষুধ খাচ্ছেন বা ইনজেকশন নিচ্ছেন	না = ০ খাবার ঔষুধ = ১ ইনজেকশন = ২	উত্তর "০" হলে ৩০ নং প্রশ্নে চলে যান

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২৮.৪	আপনি ডাইয়াবিটিস বা বহুমূত্র রোগের জন্য যে সকল ঔষুধ খাচ্ছেন বা ইনজেকশন নিচ্ছেন তার নাম বলুন ।	খাবার ঔষুধের নাম
	(অংশগ্রহনকারী ডাইয়াবিটিস বা বহুমূত্র রোগের জন্য যে ঔষুধ খাচ্ছেন বা ইনজেকশন নিচ্ছেন তা দেখুন এবং ঔষুধ বা ইনজেকশনের নাম লিখুন)	ইনজেকশনের নাম

২৯.০	**উচ্চ রক্তচাপ (ব্লাভ প্রেসার) Hypertension**		
২৯.১	আপনার উচ্চ রক্তচাপ বা ব্লাভ প্রেসার রোগ আছে কি ?	না = ০ হা = ১	
২৯.২	কত বৎসর যাবত আপনার উচ্চ রক্তচাপ বা ব্লাভ প্রেসার রোগ আছে [প্রযোজ্য না হলে = ০ ০]		
২৯.৩	উচ্চ রক্তচাপ বা ব্লাড প্রেসার রোগের জন্য আপনি কি বর্তমানে কোন ঔষুধ খাচ্ছেন ?	না = ০ হা = ১	উত্তর "০" হলে ৩১ নং প্রশ্নে চলে যান
২৯.৪	আপনি উচ্চ রক্তচাপ বা ব্লাড প্রেসার রোগের জন্য যে সকল ঔষুধ খাচ্ছেন তার নাম বলুন । (অংশগ্রহনকারী উচ্চ রক্তচাপ বা ব্লাড প্রেসার রোগের জন্য যে ঔষুধ খাচ্ছেন তা দেখুন এবং ঔষুধের নাম লিখুন)	ঔষুধের নাম	

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খাবার ও রান্নার পানির উৎসের (water source) ইতিহাস

খাবার ও রান্নার পানির উৎসের (water source) ইতিহাস			
৩০.০	আপনি কত বছর ধরে টিউবওয়েল-এর পানি পান করছেন	প্রযোজ্য না হলে = ০ ০	
৩১.০	আপনি কত বছর ধরে টিউবওয়েল -এর পানি রান্নার জন্য ব্যবহার করছেন	প্রযোজ্য না হলে = ০ ০	

খাবার পানির উৎসের তথ্য

ছয় (৬) মাসের কম সময় ধরে ব্যবহৃত খাবার পানির উৎসের তথ্য প্রয়োজন নেই

		পানির উৎস	উৎসের অবস্থান	পানির নমুনা সংক্রান্ত
৩২.১	বর্তমানে আপনি কোন উৎস থেকে সংগ্রহ করা পানি খেয়ে/পান করে থাকেন	পুকুর/নদী/কুয়া/হিন্দরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২ কত বৎসর ধরে এই উৎসের পানি খেয়েছেন/পান করেছেন	পানির উৎসের মালিক/ কেয়ারটেকার-এর নাম: যে বাড়ীতের পানির উৎসের অবস্থান সে বাড়ীর নাম নাম (যদি থাকে): বাড়ী প্রধানের নাম: বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা:	নমুনা সংগ্রহ হয়েছে (না = ০ হা= ১) পানির নমুনার আইডি নং A পানি পরীক্ষার তারিখ পানিতে আর্সেনিকের মাত্রা
৩২.২	বর্তমানে আপনি কোন উৎস থেকে সংগ্রহ করা পানি খেয়ে/পান করে থাকেন	পুকুর/নদী/কুয়া/হিন্দরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২ কত বৎসর ধরে এই উৎসের পানি খেয়েছেন/পান করেছেন	পানির উৎসের মালিক/ কেয়ারটেকার-এর নাম: যে বাড়ীতের পানির উৎসের অবস্থান সে বাড়ীর নাম নাম (যদি থাকে): বাড়ী প্রধানের নাম: বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা:	নমুনা সংগ্রহ হয়েছে (না = ০ হা= ১) পানির নমুনার আইডি নং B পানি পরীক্ষার তারিখ পানিতে আর্সেনিকের মাত্রা

HOUSEHOLD INTERVIEW SCHEDULE আইডি নং

৩২.৩	বর্তমানে আপনি কোন উৎস থেকে সংগ্রহ করা পানি খেয়ে/পান করে থাকেন	পুকুর/নদী/কুয়া/হাঁন্দরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২ কত বৎসর ধরে এই উৎসের পানি খেয়েছেন/পান করেছেন	পানির উৎসের মালিক/ কেয়ারটেকার-এর নাম: যে বাড়ীতের পানির উৎসের অবস্থান সে বাড়ীর নাম নাম (যদি থাকে): বাড়ী প্রধানের নাম: বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা:	নমুনা সংগ্রহ হয়েছে (না = ০ হা= ১) পানির নমুনার আইডি নং C পানি পরীক্ষার তারিখ পানিতে আর্সেনিকের মাত্রা
৩২.৪	বর্তমানে আপনি কোন উৎস থেকে সংগ্রহ করা পানি খেয়ে/পান করে থাকেন	পুকুর/নদী/কুয়া/হাঁন্দরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২ কত বৎসর ধরে এই উৎসের পানি খেয়েছেন/পান করেছেন	পানির উৎসের মালিক/ কেয়ারটেকার-এর নাম: যে বাড়ীতের পানির উৎসের অবস্থান সে বাড়ীর নাম নাম (যদি থাকে): বাড়ী প্রধানের নাম: বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা:	নমুনা সংগ্রহ হয়েছে (না = ০ হা= ১) পানির নমুনার আইডি নং D পানি পরীক্ষার তারিখ পানিতে আর্সেনিকের মাত্রা

খাবার পানির উৎসের তথ্য

ছয় (৬) মাসের কম সময় ধরে ব্যবহৃত খাবার পানির উৎসের তথ্য প্রয়োজন নেই

		পানির উৎস	উৎসের অবস্থান	পানির নমুনা সংক্রান্ত
৩৩.১	বর্তমানে আপার বাড়ীতে রান্নার জন্য কোন উৎস থেকে সংগ্রহ করা পানি ব্যবহার হয়ে থাকে	পুকুর/নদী/কুয়া/হাঁন্দরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২ কত বৎসর ধরে এই উৎসের পানি ব্যবহার করেছেন	পানির উৎসের মালিক/ কেয়ারটেকার-এর নাম: যে বাড়ীতের পানির উৎসের অবস্থান সে বাড়ীর নাম নাম (যদি থাকে): বাড়ী প্রধানের নাম: বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা:	নমুনা সংগ্রহ হয়েছে (না = ০ হা= ১) পানির নমুনার আইডি নং E পানি পরীক্ষার তারিখ পানিতে আর্সেনিকের মাত্রা

HOUSEHOLD INTERVIEW SCHEDULE আইডি নং

৩৩.২	এর আগে (পূর্বে) আপনি কোন উৎস থেকে সংগ্রহ করা পানি রান্নার জন্য ব্যবহার করেছেন	পুকুর/নদী/কুয়া/হাঁন্দরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২ কত বৎসর ধরে এই উৎসের পানি ব্যবহার করেছেন	পানির উৎসের মালিক/ কেয়ারটেকার-এর নাম: যে বাড়ীতের পানির উৎসের অবস্থান সে বাড়ীর নাম নাম (যদি থাকে): বাড়ী প্রধানের নাম: বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা:	নমুনা সংগ্রহ হয়েছে (না = ০ হা= ১) পানির নমুনার আইডি নং F পানি পরীক্ষার তারিখ পানিতে আর্সেনিকের মাত্রা
৩৩.৩	এর আগে (পূর্বে) আপনি কোন উৎস থেকে সংগ্রহ করা পানি রান্নার জন্য ব্যবহার করেছেন	পুকুর/নদী/কুয়া/হাঁন্দরা/বৃষ্টির পানি/ পিএসএফ এর পানি = ০ টিউবওয়েল থেকে =১ ডিপ টিউবওয়েল থেকে =২ কত বৎসর ধরে এই উৎসের পানি ব্যবহার করেছেন	পানির উৎসের মালিক/ কেয়ারটেকার-এর নাম: যে বাড়ীতের পানির উৎসের অবস্থান সে বাড়ীর নাম নাম (যদি থাকে): বাড়ী প্রধানের নাম: বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): পাড়ার/মহল্লার নাম (যদি থাকে): গ্রাম: ওয়ার্ড : মৌজা: ইউনিয়ন: উপজেলা: জেলা:	নমুনা সংগ্রহ হয়েছে (না = ০ হা= ১) পানির নমুনার আইডি নং G পানি পরীক্ষার তারিখ পানিতে আর্সেনিকের মাত্রা

১. নির্বাচিত অংশগ্রহণকারীর **Household** ফর্ম পূরণ শেষে প্রয়োজনীয় তথ্য অংশগ্রহণকারী লগ-এ এখন লিপিবদ্ধ করুন ।

২. **Household interview schedule**-এর সাথে অংশগ্রহণকারীর স্বাক্ষর / টিপসহি যুক্ত সম্মতি পত্র সংযুক্ত করুন ।

৩. যে অংশগ্রহণকারীর জন্য **Household interview schedule** সম্পন্ন করা হয়েছে তাকে রেফারেল স্লিপ প্রদান করুন এবং তার কি করণীয় তা বুঝিয়ে দিন ।

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh.

MEDICAL EXAMINATION CHECKLIST

আইডি নং ☐☐☐☐☐

Medical examination এর জন্য registration কৃতদের ক্ষেত্রে এই ফর্ম পুরন করুন ।

উপজেলা _____ জেলা _____

Registration- কারীর নাম ও স্বাক্ষর

নাম _____

স্বাক্ষর _____ তারিখ _____

1	অংশগ্রহণকারী সকালে খেয়ে এসেছেন **(fasting)**	না = ০ হা = ১	☐
2	অংশগ্রহণকারী ডাইয়াবিটিস বা বহুমূত্র রোগের জন্য ঔষুধ খাচ্ছেন বা ইনজেকশন নিচ্ছেন কি ? **(dmmedic)**	না = ০ হা = ১	☐
যে সকল অংশগ্রহণকারী ডাইয়াবিটিস বা বহুমূত্র রোগের জন্য ঔষুধ খাচ্ছেন বা ইনজেকশন নিচ্ছেন তাদের ক্ষেত্রে **blood glucose** নির্ণয়ের প্রয়োজন নাই			

Fasting Blood capillary glucose estimation

3	Fasting capillary blood glucose মাত্রা **cbgl1**	☐☐☐☐
4	After determination of capillary blood glucose allow the participant to drink the glucose solution (82.5 g of glucose monohydrate in 300 ml of water) over the course of 5 minutes under direct supervision.	Time of when the glucose drink was taken ☐☐ ☐☐ AM / PM
5	**Record the time when the drink was completed and the time when the participant was expected to have the 2nd Fasting capillary blood glucose estimation (2 hours after the glucose drink)**	Expected time for 2nd CBG estimation ☐☐ ☐☐ AM / PM

এখন অংশগ্রহণকারীকে **Anthropometric assessment** জন্য পাঠান

ANNEX-VII

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh.

MEDICAL EXAMINATION CHECKLIST

আইডি নং

Anthropometric assessment

6	ওজন [weight (kg)] (**wt**)		
7	উচ্চতা [height (cm)] (**ht**)	cm	.
8	BMI (গবেষক নির্ণয় করিবেন) (**bmi**) weight /(height)^2=	kg/m^2	.

Anthropometry শেষে রক্ত চাপ (**Blood Pressure**) পরিমাপ এর জন্য পাঠান

9	অংশগ্রহণকারী উচ্চ রক্তচাপ বা ব্লাড প্রেসার রোগের জন্য কোন ঔষুধ খাচ্ছেন কি ? ***htnmed***	না = ০ হা = ১	

যে সকল অংশগ্রহণকারী উচ্চ রক্তচাপ বা ব্লাড প্রেসার রোগের জন্য ঔষুধ খাচ্ছেন তাদের ক্ষেত্রে রক্তচাপ বা ব্লাড প্রেসার নির্ণয়ের প্রয়োজন নাই

Blood pressure assessment

10	Blood pressure to be measured in (left arm used only when rt arm is absent, paralysed or deformed) (**bparm**)	Right arm = 0 Left arm = 1	
11	Greatest circumference of the right arm measured, with the arm relaxed and in the normal blood pressure measurement position (**bparmcur**)	(measured to the nearest 0.1 cm.)	
12	Cuff selected (based on arm circumference) (25 cm - 35 cm: use adult cuff over 35 cm: use large adult cuff) (**cuffused**)	Adult cuff = 0 Large Adult cuff = 1	

Blood pressure measurements right arm

		I	II	III	Average of the closest pair of the readings
13	Systolic (SBP) mm Hg	**sbp1**	**sbp2**	**sbp3**	**sbp** (to be calculated by researcher)
14	Diastolic (DBP) mm Hg	**dbp1**	**dbp2**	**dbp3**	**dbp** (to be calculated by researcher)

রক্ত চাপ (Blood Pressure) পরিমাপ শেষে অংসগ্রহণকারীকে **General Medical Examination** এবং **ABI assessment** এর জন্য পাঠান

Arsenic in drinking water and Lower extremity arterial disease in Bangladesh.

MEDICAL EXAMINATION CHECKLIST

আইডি নং

General Medical Examination (to be carried out by a physician)

15	Anemia ***anaem***	No = 0 Mild = 1 Moderate= 2 Severe=3	
16	Non purulent conjunctival congestion ***npccog***	No = 0 Yes = 1	
17	Jaundice ***jaun***	No = 0 Yes = 1	
18	Central cyanosis ***ccyano***	No = 0 Yes = 1	
19	Peripheral cyanosis ***pcyano***	No = 0 Yes = 1	
20	Clubbing ***clubb***	No = 0 Yes = 1	
21	Oedema (pedal) ***pededem***	No = 0 Non-pitting = 1 Pitting = 2	
22	Dehydration ***dehyd***	No = 0 Mild = 1 Severe=2	
23	Palpable liver ***pliver***	No = 0 Yes = 1	
24	Palpable spleen ***pspleen***	No = 0 Yes = 1	
25	Palpable intra-abdominal mass ***piamass***	No = 0 Yes = 1	
26	Melanosis on the trunk **(both on front & back)** ***mtrunk***	No = 0 Yes = 1	
27	Keratosis on both palms & soles ***kpsole***	No = 0 Yes = 1	
28	Amputation of limb or part ***ampu***	No = 0 Yes = 1	
29	Site of amputation ***aplimb*** Rt Limb =0 Left limb = 1	1 or more fingers or digits of finger(s) of lower limb = 0 Mid tarsal = 1 Below knee = 2 Mid thigh = 3 1 or more fingers or digits of finger(s) of upper limb = 4 Mid carpal = 5 Below wrist = 6 Below elbow = 7 Mid arm = 8 **lamp**	
30	Nature of amputation ***natamp***	Spontaneous – 0 Surgical = 1 Accidental = 2	
31	Gangrene of lower limbs(s) ***gangll***	No = 0 Yes = 1	
32	Location of gangrene of lower limbs(s) ***locgang***	finger(s) of right foot = 0 finger(s) of left foot = 1 part of right foot beyond finger(s) = 2 part of left foot beyond finger(s) = 3	

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MEDICAL EXAMINATION CHECKLIST

আইডি নং | | | | | |

Assessment of ABI

33	Right Brachial artery systolic pressure *mm Hg*	***rbsp***	Highest of the 2 Brachial artery pressures *mm Hg* **hbsp**
34	Left Brachial artery systolic pressure *mm Hg*	***lbsp***	
35	Left tibialis posterior artery systolic pressure *mm Hg* (***ltpsp***)		Highest left ankle pressure **HLASP**
36	Left dorsalis pedis artery systolic pressure *mm Hg* (***ldpsp***)		
37	Right tibialis posterior artery systolic pressure (***rtpsp***) *mm Hg*		Highest right ankle pressure **HRASP**
38	Right dorsalis pedis artery systolic pressure (***ldpsp***) *mm Hg*		

Post glucose load Capillary blood glucose estimation

39	2nd capillary blood glucose estimation এ glucose এর মাত্রা **cbgl2**	.

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অংশগ্রহণকারী লগ

১. আর্সেনিকোসিস (arsenicosis) গ্রুপে নির্বাচিত অংশগ্রহণকারীর বিপরীতে অন্যান্য গ্রুপের জন্য একই লিঙ্গের অংশগ্রহণকারী (পুরুষ হলে পুরুষ) নির্বাচন করতে হবে ।

২. অন্যান্য গ্রুপের জন্য অংশগ্রহণকারী নির্বাচনের সময় নির্বাচিত্য অংশগ্রহণকারীর বয়স আর্সেনিকোসিস (arsenicosis) গ্রুপে নির্বাচিত অংশগ্রহণকারীর বয়সের ± ২ বৎসর মধ্যে হতে হবে, তবে কোনভাবেই ৩০ বৎসরের কম বা ৬০ বেশী হবে না ।

আর্সেনিকোসিস (arsenicosis) গ্রুপ গ্রুপ -১				আর্সেনিকোসিস ছাড়া আর্সেনিক একস্পোজার গ্রুপ (Arsenic exposure group without arsenicosis) গ্রুপ -২				রেফারেন্স গ্রুপ [Reference Group] গ্রুপ -৩			
Sl no	আই ডি নং	লিঙ্গ	বয়স (বৎসর)	Sl no	আই ডি নং	লিঙ্গ	বয়স (বৎসর)	Sl no	আই ডি নং	লিঙ্গ	বয়স (বৎসর)
		পুরুষ মহিলা				পুরুষ মহিলা				পুরুষ মহিলা	
		পুরুষ মহিলা				পুরুষ মহিলা				পুরুষ মহিলা	
		পুরুষ মহিলা				পুরুষ মহিলা				পুরুষ মহিলা	
		পুরুষ মহিলা				পুরুষ মহিলা				পুরুষ মহিলা	
		পুরুষ মহিলা				পুরুষ মহিলা				পুরুষ মহিলা	
		পুরুষ মহিলা				পুরুষ মহিলা				পুরুষ মহিলা	
		পুরুষ মহিলা				পুরুষ মহিলা				পুরুষ মহিলা	

প্রয়োজনীয় তথ্য অংশগ্রহণকারী লগ-এ এখন লিপিবদ্ধ করার পর রেফারেল স্লিপ পূরণ পূর্বক অংশগ্রহণকারী প্রদান করুন এবং পরবর্তীতে কি করণীয় তা বুঝিয়ে দিন

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রেফারেল স্লিপ

আই ডি নং | | | | | |

(১) অংশগ্রহণকারীর পরিচিতি

নাম: _____ বয়স _____ পুরুষ/ মহিলা

বাবার নাম: _____

স্বামীর নাম (প্রযোজ্য ক্ষেত্রে): _____

(২) বর্তমান বাসস্থান সম্পর্কিত

বাড়ীর (খানা) নং {জিআর নং} (যদি থাকে): _____

বাড়ীর নাম (যদি থাকে): _____

পাড়ার নাম (যদি থাকে): _____

গ্রাম: _____ ওয়ার্ড : _____ ইউনিয়ন: _____

মৌজা: _____ ** উপজেলা: _____ **জেলা: _____

১. অংশগ্রহণকারী ডাইয়াবিটিস বা বহুমত্র রোগের জন্য বর্তমানে ঔষুধ খাচ্ছেন বা ইনজেকশন নিচ্ছেন ? হা / না

২. অংশগ্রহণকারী উচ্চ রক্তচাপ বা ব্লাড প্রেসার রোগের জন্য বর্তমানে ঔষুধ খাচ্ছেন ? হা / না

আগামী _____ তারিখ _____ বার সকাল _____ ঘটিকায় কেন্দ্রে /নির্ধারিত স্থানে (নাস্তা না খেয়ে) স্বাস্থ্য পরীক্ষার জন্য উপস্থিত হবার জন্য আপনাকে অনুরোধ জানানো হচ্ছে ।

রেফারকারীর নাম _____

রেফারকারীর স্বাক্ষর _____ তারিখ _____