Impact of Introducing High-Selenium Lentils on Everyday Diet for Mitigating Arsenic Related Health Problems in Bangladesh

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By

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To my Parents and Family

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Abstract

Background: Chronic arsenic (As) toxicity (arsenicosis) due to consumption of ground water polluted with higher level of As is a major global environmental problem. Worldwide, above 100 million people are found to be chronically exposed to As through drinking water. Despite major international and national efforts to reduce As contamination in drinking water, millions of Bangladeshis are still at risk of high As exposure via consumption of As contaminated tube-well water as well as food. The micronutrient selenium (Se) is known to be an essential element for humans. Selenium has been suggested to counteract As-induced toxicity through its antioxidant properties by promoting the excretion of As from the body. Low blood Se level increases the risk of As-induced health problems such as skin lesions, cancers in different organs, black foot disease (an exceptional peripheral vascular disease affects the lower extremities of the body) etc. It has also been reported that Bangladeshis have a low intake of Se due to the deficiency of Se in Bangladeshi soil similar to many parts of the world. Therefore, crops grown in Bangladesh contain a low level of Se. Lentils grown in western Canada, especially in Saskatchewan, are rich in Se mainly in the form of L-selenomethionine, which is exclusively bioavailable. Incorporation of these high Se lentils into the daily meals of As-exposed Bangladeshi families has the potential to reduce As-induced morbidity without having to change people's food habit as Bangladeshis eat lentils daily as "dahl." The beneficial impact of high Se lentils to mitigate As induced toxicity has been shown in a number of experimental studies, including various animal models like rats, mice, and rabbits. In several human trials, investigators also used Se pills to reduce As toxicity-related health problems.

Hypothesis: In this study, we hypothesized that the consumption of high-Se containing lentils would decline the body load of As and reduce As exposure related toxicity in a chronically exposed Bangladeshi population compared to those consuming low-Se lentils.

Aim: The aim of this thesis was to demonstrate if the daily intake of lentils, naturally bio-fortified with Se for six months could result in health benefits in an As-exposed rural Bangladeshi population who had been chronically exposed to As through contaminated drinking water.

Method: This thesis was a part of a randomized, double-blinded, placebo-control, community-based supplementation trial conducted in Shahrasti, a rural area of Bangladesh where people are frequently exposed to a very high level of As (>100 ppb) through their tube well water. A total of 405 participants chronically exposed to As were enrolled in the trial. Each participant was taken 65 g of lentils per day during the intervention period of 6 months. Participants from the intervention arm (Se-group) consumed Se-rich lentils (55µg Se/day, the recommended dietary allowance (RDA) dose of Se) while from the control group received placebo lentils of similar nutrient profile except with low Se (1.5µg Se/day). Anthropometric measurements (height, weight, blood pressure), blood, urine, hair and stool samples were collected at baseline, month 3(except for hair) and end of the intervention at month 6. Morbidity data were collected fortnightly. Total urinary As (U-As, specific gravity adjusted), stool-As (subsample, n= 132), and hair-As from all the collected samples were measured by hydrogen vapor generated atomic absorption spectrometry (HVG-AAS) while urinary Se (U-Se) at all the three-time points, erythrocyte As (Ery-As), erythrocyte Se (Ery-Se) at baseline and month 6 were measured by inductively coupled plasma mass spectrometry (ICP-MS). The fractionated nitric oxide level (FE_{NO}) in exhaled air was measured at the three-time points to assess lung inflammation by NIOX MINO. Total glutathione content in erythrocytes (Ery-GSH) was measured by recycling assay method, and plasma levels of

8-hydroxy-2'-deoxyguanosine (8-OHdG) were analyzed by a competitive immunoassay by ELISA. Plasma C-reactive protein and lipids levels were measured by a biochemistry analyzer.

Results: Consumption of Se bio-fortified lentils resulted in increased urinary As excretion ($p=0.001$), improved body mass index ($p\leq0.01$), and reduced frequency of asthma ($p=0.05$) and allergy ($p=0.02$) among the study participants in the intervention arm (Se-group) compared to those in the control arm (control group). It was also linked with higher levels of Se in the erythrocyte ($p <0.001$). Recommended dietary allowance dose of Se supplementation through lentils did not alter the levels of oxidative stress, antioxidant defense, lung inflammatory marker (FE_{NO}) and the biomarkers of cardiovascular diseases in the current population, while lentil supplementation for six months reduced lung inflammation and regulate biomarkers of cardiovascular diseases (particularly blood pressure and plasma lipids except for triglycerides) after six months of supplementation.

Conclusion: Daily consumption of RDA dose of Se through lentils for six months can increase As excretion, blood Se content and thereby improve some of the health indicators, while lentil supplementation regardless of Se content can provide a number of health benefits to the chronically As exposed rural Bangladeshi population.

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8 OHdG	8-hydroxy-2'-deoxyguanosine
As	Arsenic
BP	Blood pressure
BMI	Body mass index
Chol	Cholesterol
CVD	Cardiovascular diseases
CRP	C-reactive protein
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
DBP	Diastolic blood pressure
Ery-As	Erythrocyte arsenic
Ery-Se	Erythrocyte selenium
Ery-GSH	Total glutathione content in erythrocytes
FE_{NO}	Fractionated nitric oxide level
GEE	Generalized estimating equation model
TG	triglyceride
GSH	Total glutathione
HH	Households
HVG-AAS	Hydrogen vapor generated atomic absorption spectrometry
HDL	High density lipoprotein
ICP-MS	inductively coupled plasma mass spectrometry
LDL	low density lipoprotein
MPA	Meta-phosphoric acid
NIST	National Institute of Standards and Technology
ROS	Reactive oxygen species
RDA	Recommended dietary allowance
SBP	Systolic blood pressure
Se	Selenium
SES	Socioeconomic status
TNB	2-nitro-5-thiobenzoate anion
U-As	Urinary arsenic
U-Se	Urinary selenium

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Chapter 1

INTRODUCTION

This thesis focuses on the effects of Se-rich lentil supplementation to mitigate chronic arsenic (As) exposure associated with health problems in the rural Bangladeshi population consuming highly As-contaminated drinking water. Chronic As toxicity (arsenicosis) through the consumption of high As containing groundwater is a major environmental health hazard throughout the world. Groundwater As contamination is prevalent in many countries, including Bangladesh, parts of Asia, Central Europe, Chile, Mexico, and the United States, where As in water exceeds the World Health Organization's (WHO)recommended value of 10 μg/L (1).Despite several major international and national initiatives to reduce groundwater As contamination, very little progress has been noticed in Bangladesh. The adverse health effects of As poisoning are aggravated with malnutrition or the consumption of low micronutrient containing foods. Selenium (Se), an essential trace nutrient that antagonizes many of the adverse effects of As through its antioxidant properties in biological systems(2). Selenium deficiency exacerbates clinical symptoms of As toxicity (arsenicosis) in humans(3). Lentil is one of the staple foods consuming daily by the Bangladeshi people. The form (L-selenomethionine) of Se present in lentils is highly bioavailable (4, 5). Selenium content in diet is dependent on soil Se level. The agricultural soil of Bangladesh is Se deficient (6). Thus lentils grown in Se rich soil of Saskatchewan, Canada, could be a good source of Se. This trail investigates the impact of Se rich lentil supplementation on reducing the body burden of As in the exposed Bangladeshi population.

1.1 Arsenic

Arsenic (As) is a natural element found ubiquitously in the environment, cycling through water, land, air, and living systems with tremendous implications for human exposure, especially drinking water. It is a well-recognized carcinogen. It is considered as one of the world's most hazardous chemicals(7) and a metalloid that can show both metallic and non-metallic properties with atomic number 33, relative atomic mass 74.92, and electronic configuration 4s23d104p3.In terms of abundance, As ranked the 20th most common element in the earth's crust and soil, it presents at the level of 2–5 mg/kg (8, 9). A number of combined natural processes including weathering reactions, biological activity, and volcanic emissions, and through a wide range of anthropogenic actions usually mobilize As(10). The two forms, arsenite (As^{III}), and arsenate (As^{V}), are the main oxidation states in reducing and oxygenated settings, , respectively (11, 12). Arsenic compounds are divided into three major groups:

1. Inorganic As compounds (As trioxide, sodium arsenite, As trichloride, As acid, and As pentoxide),

2.), and Organic As compounds (arsanilic acid, monomethyl arsonic acid, dimethylarsinic acid, arsenobetaine, and arsenosugars

3. Arsine gas.

The most common inorganic forms of As in water are arsenite and arsenate, while arsenobetaine, arsenocholine, and arsenosugars are the important organic forms in certain foods, particularly in seafood (11).Inorganic As is more toxic than organic As.

1.2 Usage of arsenic

Arsenic is mostly used as an insecticide and herbicide or preservative for wood with its germicidal power and resistance to rotting and decay. In medicine, electronics, and industrial manufacturing(13), As is also used. Organic arsenicals are used to increase the rate of weight gain for poultry and swine (14). In the manufacture of alloys, metallic As is used, mainly with lead (e.g., in lead-acid batteries) and copper. The light-emitting and electromagnetic properties of gallium arsenide are used in high-speed semiconductor devices (15). In computer chips and fiber optics, arsine gas is used as a doping agent (16).

1.3 Sources of arsenic exposure

Arsenic is a trace element primarily found in the earth's crust and groundwater. Air and food items (crustaceans and seafood) also contain low level of As (17). Groundwater is one of the largest sources of As poisoning worldwide. Crops grown in As-contaminated soils and/or irrigated with As-contaminated water are the main sources of As exposure from ingested foods. Groundwater is the primary source of As exposure in Bangladesh where people unconsciously consume higher levels of As through the tube well water while using it daily for drinking and cooking purpose. Around 150 million people globally is likely to be affected to As exposure, with an increasing rate as newly affected areas are continuously discovered (18). **Figure 1.1** shows a global As map with a number of people at risk.

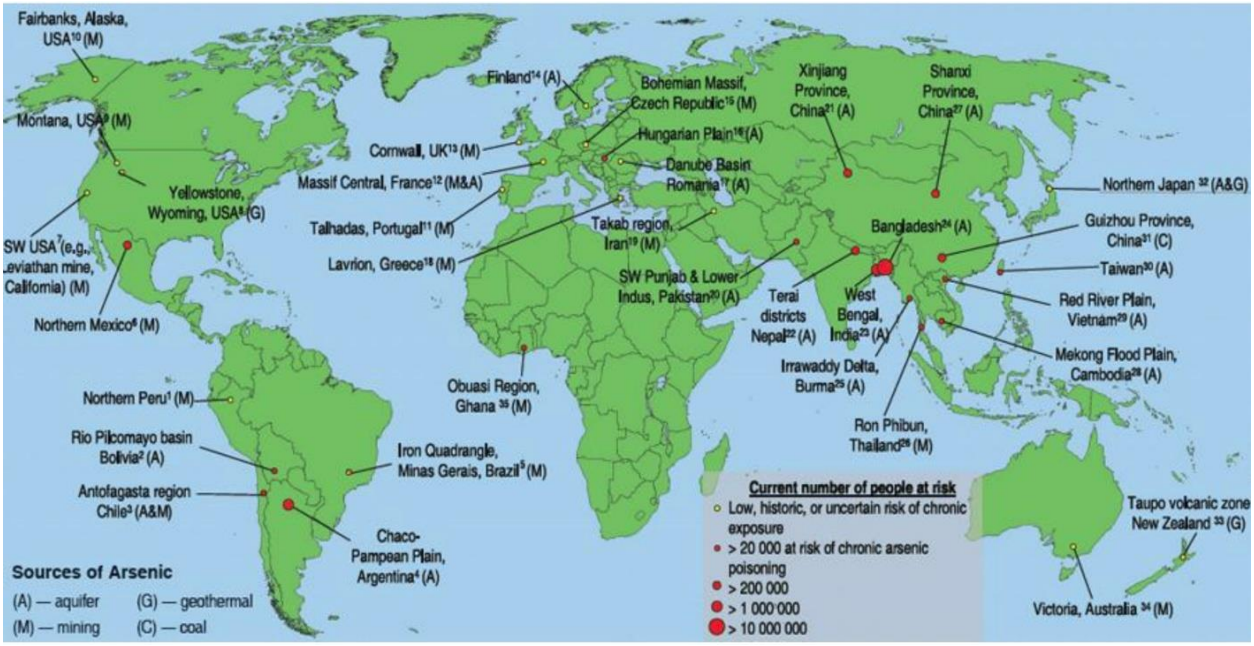

Figure 1.1 Universal spreading of arsenic-contaminated areas, showing the source of arsenic and numbers of people at risk of chronic exposure. Figure adapted from (Revenscroft, P, 2009) (8).

1.4 Groundwater arsenic contamination

Groundwater As contamination (caused by natural and/or anthropogenic sources) is globally an alarming problem(19). For drinking purposes, a large number of people from different regions of the world are extensively dependent on ground water for daily consumption, even though the ground water from those areas are containing elevated levels of As and thereby people are being exposed to higher levels of As. Many countries, particularly developing ones, including Bangladesh, still use groundwater containing > 50 µg/L of As(national standard level in Bangladesh) because of limitation of testing and shortage of As removal facilities for lowering As concentrations that requires financial support. Ground water As pollution is found in Chile, Mexico, China, Argentina, USA, and Hungary (20, 21)as well as in Asia, especially in the Indian State of West Bengal,

Bangladesh and Vietnam (19, 20, 22-25). **Table 1.1** shows the levels of As contamination indifferent As affected countries with the permissible limits

Table 1.1 Arsenic in natural groundwater in various countries

Serial number	Country	Groundwater As level (µg/L)	Permissible limit (ppb)
1	Afghanistan	10–500	10 (WHO)
2	Bangladesh	<1–4730	50 (WHO)
3	Brazil	0.4–350	10 (WHO)
4	Canada	1.5–738.8	10 (WHO)
5	China	50–4440	50 (WHO)
6	Finland	17–980	10 (WHO)
7	India	10–3200	50 (WHO)
8	Japan	1–293	10 (WHO)
9	Mexico	8–620	25
10	Nepal	Up to 2620	50
11	Pakistan	Up to 906	50
12	Taiwan	10–1820	10 (WHO)
13	Thailand	1–>5000	10 (WHO)
14	USA	Up to 2600	10 (USEPA)
15	Vietnam	<1–3050	10 (WHO)

Adapted from (19)

1.5 Arsenic contamination in Bangladesh

Bangladesh is positioned on the largest deltaic land in the world with mostly low-lying floodplain areas containing about 75% of the land being less than three meters above the sea level. Ganga–Brahmaputra–Meghna (GBM) river system forms the Bengal Basin where Bangladesh is mainly located. This Bengal Basin is a sedimentary basin that has been formed by the deposition of large volumes of As-containing sediments originated

mainly from the Himalayas. The As-containing sediments in the Bengal Basin are carried down by the mighty GBM rivers, contaminating the surface and underground water which is the main source of drinking water in Bangladesh(26). Before initiating the tube-wells, people in Bangladesh were mainly dependent on surface water sources like rivers, canals, lakes, ponds, and ring wells. However, majority of these water sources were contaminated with various microorganisms that consequently increase the incidence of diarrheal diseases and cholera. Thus, during the seventies in rural Bangladesh, United Nations International Children's Emergency Fund (UNICEF), the government of Bangladesh and a number of private companies, dug millions of tube wells so that the mortality rate of water borne diseases including cholera and other diarrheas had been reduced(27, 28). The initiative of digging thousands of tube-well, accompanied by the awareness programs, had a great success which substantially brought down the occurrence of diarrheal diseases and cholera. However, the detection of As pollution in the tube-well water and its impacts on human health among the exposed people lead to the reversal of the success story (29-31).

The first survey in Bangladesh to detect As pollution was conducted in 1993(32) in northern bordering areas. Then millions of hand-pumped tube-wells were found to yield drinking water containing a higher level of As, above WHO recommended value (<10 μg/L).moreover several tube wells were polluted with As ranging from the levels of 59-388 μg/L which was higher than Bangladesh national standard level for drinking water <0.05mg/L(33-36). In 2000, Karim et al. showed that about 40 million Bangladeshi people from 52 districts out of 64 were at risk of As contamination which caused As induced common diseases to the affected people, i.e., melanosis, keratosis, hyperkeratosis, dorsum, gangrene, and skin cancer (37).In 2002, the World Health

Organization (WHO) termed the case of Bangladesh as “the world’s largest mass poisoning of a population in history” (38). The annual treatment cost for the health problems, associated with chronic As exposure among Bangladeshis has been predicted at up to $77.5million(1). It is also estimated that, daily average 1017.0 µg of As has been consumed by the people living in contaminated areas, per capita. It has been reported that on daily basis, a Bangladeshi male living in an As-contaminated area, daily consumes 1734µg of As on an average through drinking water while a female ingests 1321µg of As; they also get additional exposure through vegetables and grains that they consume (39).

Exposure to As can cause skin lesions and other long-lasting diseases such as diabetes mellitus, hypertension etc.(40). The toxicity of As to the human body varies depending on several factors such as general health and diet. It is estimated that, per year about >9000 people died from As-related diseases in Bangladesh(41), while in another study among the patients with skin lesion from Pabna, Jessore, Kustia, Chuadanga, Narayanganj, and Meherpur districts found 16.6% of the patients had died due to cancer since 1996-2009(42).

Even though various organizations have undertaken various mitigation measures, majority of the actions could not achieve their expected results and remained unsuccessful due to technical, spatial, and socio-economic challenges. Mitigation of As contamination is a complex issue requiring a coordinated effort from various levels of stakeholders (43). Thus, other mediations need to be applied to mitigate As-related health problems.

1.6 Metabolism of arsenic

In the contaminated groundwater, As is primarily found in inorganic forms (44). In most mammals, ingested inorganic As is generally metabolized by methylation which occurs via

alternating reduction of pentavalent As to the trivalent state, followed by oxidative addition of a methyl group (45-48). In biological system, ingested inorganic As is bio-transformed mainly in liver(49) and 70% metabolites generated via the bio-transformation process excreted readily through urine (50-53). The left over fraction of As stores divergently in other organs and tissues for example kidneys and lungs, along with keratinized tissues such as skin, nails, and hair (54, 55).Therefore, As methylation is extensively considered as a way of bio-activation instead of detoxification (56). The severity of As toxicity is associated to the methylation status and valence state of the metabolites (57). Indeed, inefficient As methylation has been associated with increased As contamination causing As-induced skin lesions, cancer, and cardiovascular effects (58-60). Arsenic methylation can be modified by nutritional status which affects the one-carbon metabolism (61, 62). Thus, the adverse effect of As exposure is exacerbated in acute malnutrition (63, 64) which is very common health problem among Bangladeshi population.

1.7 Impact of chronic arsenic exposure on human health

Chronic As exposure is not only associated with cancers in different organs (skin, lung, bladder, kidney, liver) (11), but also linked with various non-cancerous health complications, including skin diseases (65), diabetes (66), cardiovascular disease (67), immune-toxicity (68-70) and impaired child development (71-73).Arsenic is also known to cause cytotoxicity (74, 75) and genotoxicity (76)(**Figure 1.2**). Chronic As exposure is also known to be associated with adverse pregnancy outcomes and infant mortality, impacting child health, and there is some records of negative impacts on cognitive development (77).A number of experimental studies have shown that As toxicity is mediated by induction of oxidative stress (54). The formation of reactive oxygen species (ROS) i.e.

superoxide anion radical and hydrogen peroxide have been increased in response to As exposure (78). Oxidative stress and inflammation cause various chronic diseases such as, cancer, cardiovascular diseases (CVD), and diabetes (79, 80). In a longitudinal study among Bangladeshi pre-adolescent children, we showed that chronic As exposure from in utero to adolescent period altered the levels of biomarkers (i.e., blood pressure, plasma cholesterol, LDL, HDL, TG, glucose, etc.) of cardio-metabolic diseases (81).

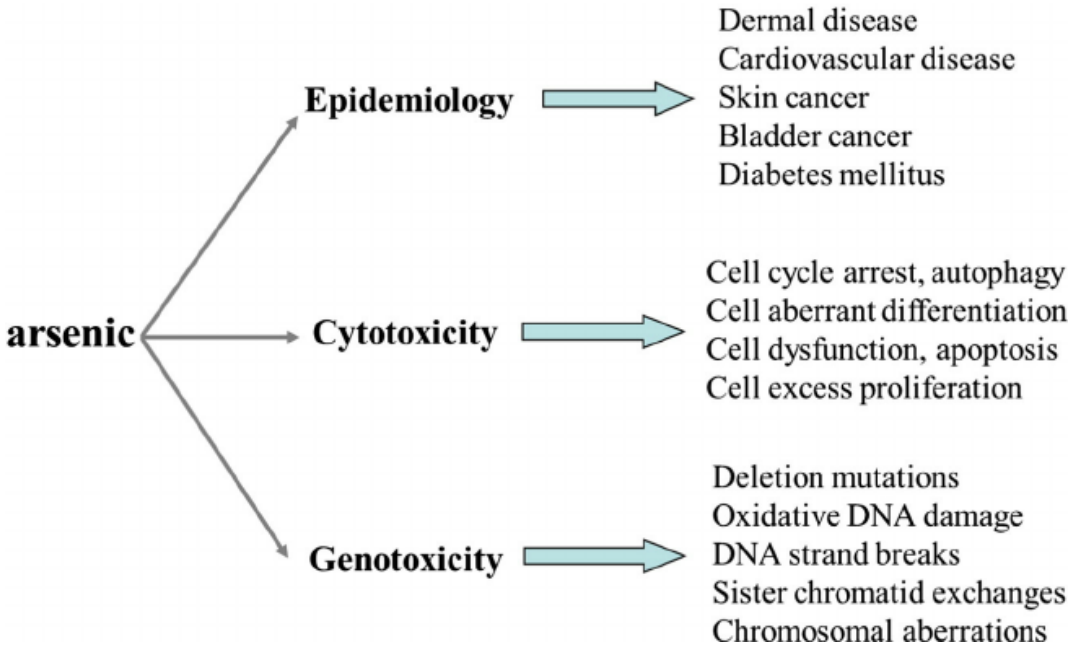

Figure 1.2 Arsenic toxicity in human, adapted from (82)

1.8 Selenium and its role in human health

The micronutrient selenium (Se) is a vital non-metal trace element for biological system of human and animal (83). With its antioxidant properties, Se itself performs crucial role in immune system, cancer prevention. Selenium facilitates numerous activities through seleno- proteins, which keep the cells protected from oxidative and inflammatory damage and regulate immune function (66, 84, 85).The first biological role of Se was recognized in

1973 when it was revealed to be an integral component of erythrocytes' glutathione peroxidase (GPx), which is a powerful antioxidant enzyme (86, 87).

Selenium is used as a critical component of several major metabolic pathways. Selenium also has anti-inflammatory (88, 89) anti-mutagenic (90), anti-carcinogenic(91, 92), or chemo-preventive (93, 94), antiviral (95), antibacterial (96, 97) capacity. Being an intrinsic part of seleno-proteins, Se exerts its role in the series of physiologically important processes(98), including synthesis (95, 99), metabolism(85, 99, 100) and function of thyroid hormones (101), that are critically regulate the process of development, growth, and differentiation. Selenium was found to be beneficial for the patients having inflammatory as well as infectious diseases. In chronic inflammation it was found that, Se intervention restored decreased serum and hepatic tissue Se levels by increasing the synthesis of seleno-protein, thereby decrease CRP production and altering the inflammatory process (89).Deficient Se level is linked with increased risk of different chronic diseases, particularly viral infections, thyroid function alterations, and CVDs(102).

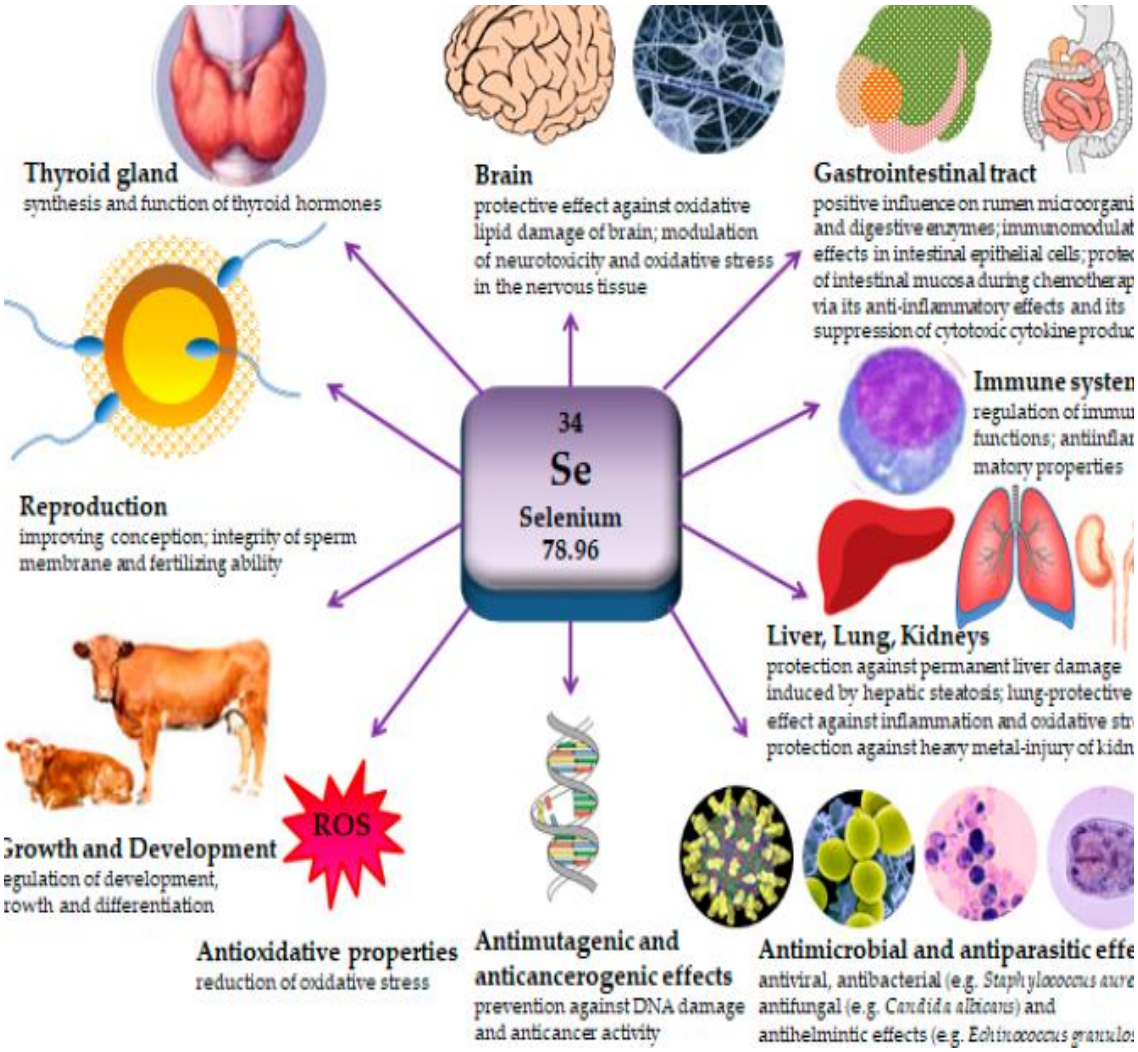

Figure 1.3 Physiological effect of selenium, adapted from (103)

1.9 Beneficial effects of selenium to counteract arsenic induced toxicity

Selenium is an essential trace element for the biological system having antioxidant properties and playing critical role in the immune system. It is a known antagonist to inorganic As. (104, 105). The antagonism of Sein As toxicity is well established(104-109). Animal experiments showed that Se could increase the excretion of As through bile(107)as well as via urine (110). Chronic daily exposureto200-250 µg of inorganic As will ultimately result in cancer in humans (111). Whereas, a daily intake Se, in the dose of

between 50 and 200 μg is suggested to deliver humans with an sufficient supply of this metalloid to produce vital seleno-proteins(112).

Animal studies have demonstrated that, the intravenous injection of Se formed a complex with As [selenobis-(S-glutathione) arsinium anion $[(GS)_2AsSe]^-$that excreted via bile into the intestinal tract (113-115). Experimental studies in rodents have also confirmed that, high Se lentils diet decreased As content in tissue, As-induced hepatic damage and increased antioxidant levels(109, 116).A number of experimental and animal studies has been demonstrated the interaction of As and Se and revealed that when As and Se are taken or injected together, maximum proportion of As and Se are moved to the liver where As–Se form a compound $[(GS)_2AsSe]^-$using glutathione (GSH). This complex is relatively less toxic and causes reduced amount of damage on cells than As or Se alone(113, 114, 117, 118). Thus ingestion of Se and As simultaneously facilitating the excretion of each other in bile from hepatocytes.

In the biological system of the patients with chronic As exposure, Se can play beneficial role as a potential therapeutic agent through antagonizing many detrimental effects of As toxicity(105, 119). People with low blood Se levels found to be more susceptible for developing As-related skin lesions (120).In context of above, a number of Se supplementation trials have been conducted to treat chronic As poisoning (105, 106, 108, 109, 120-122).A cross-sectional study among adults in Araihazar, Bangladesh showed inverse association of plasma Se with urinary, blood As concentrations and genomic DNA methylation (123). Another study showed that the participants with comparatively low blood Se levels (> the average, 150.2 μg/L) were constantly remained at a higher risk of As exposure associated skin lesions (124).The main protection mechanism of Se against As

pollution in human is based on sequestration of As into biologically inert complexes through the action of Se-dependent antioxidant enzymes(82, 125). To reduce As accumulation after chronic As exposure, Se supplementation can be a better approach.

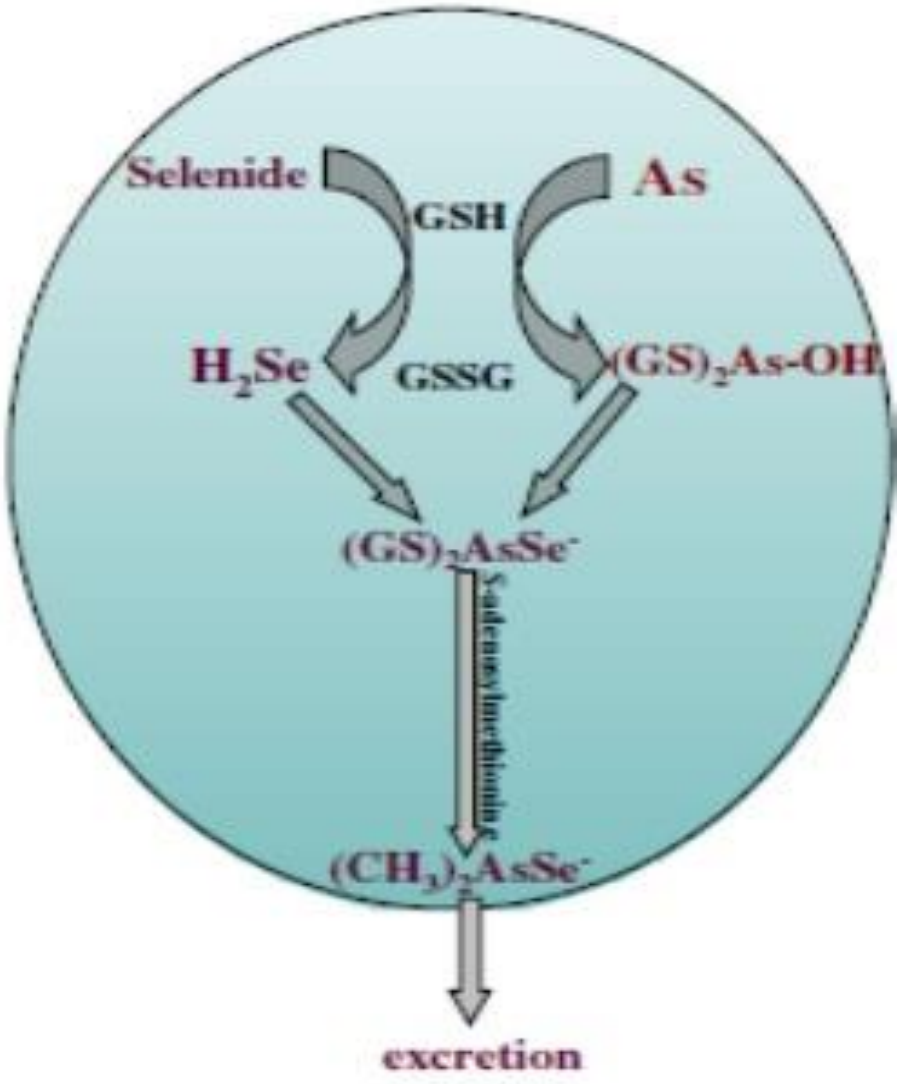

Figure 1.4. Antagonistic effect of Se and As, adapted from (82)

1.10 Dietary sources of selenium

Selenium content in the soil maintains the bioavailability of Se in the food crops. The concentration of Se in natural food is dependent on the content of Se in the soil. Very few information is available regarding Se content in Bangladeshi soil. In a previous study, it has been found that in Bangladesh the agricultural soils (n= 19) contained sufficient levels of Se(126). However, it has been shown that only 12% of soil Se was soluble that reduce the available Se concentration to 0.12 μg/g. A "nutritional selenium deficiency" in the soil is <0.5 μg/g soil (6); so Bangladeshi soil is being in the Se deficient category.

In the United Kingdom (UK), the recommended reference nutrient intake (RNI) of Se is 60 μg/day for adult women, 75 μg/day for lactating women and adult men (127). For the US men and women, the daily recommended intake is 55μg(128). The European Food Safety Authority (EFSA) in European Union has set an adequate daily intake for Se at 70 μg(129). It is estimated that about 26 μg/day of Se is consumed by the people in Bangladesh(126),which is approximately half of the US daily recommended intake (55 μg/day). The optimal intake of Se to achieve additional health benefits to maintain good health and the best bio marker(s) to determine Se sufficiency have yet to be established conclusively (130). Selenium can be obtained from both animal and plant sources. The wide variety of Se containing foods include grains, vegetables, seafood, meat, dairy products, and nuts (131).

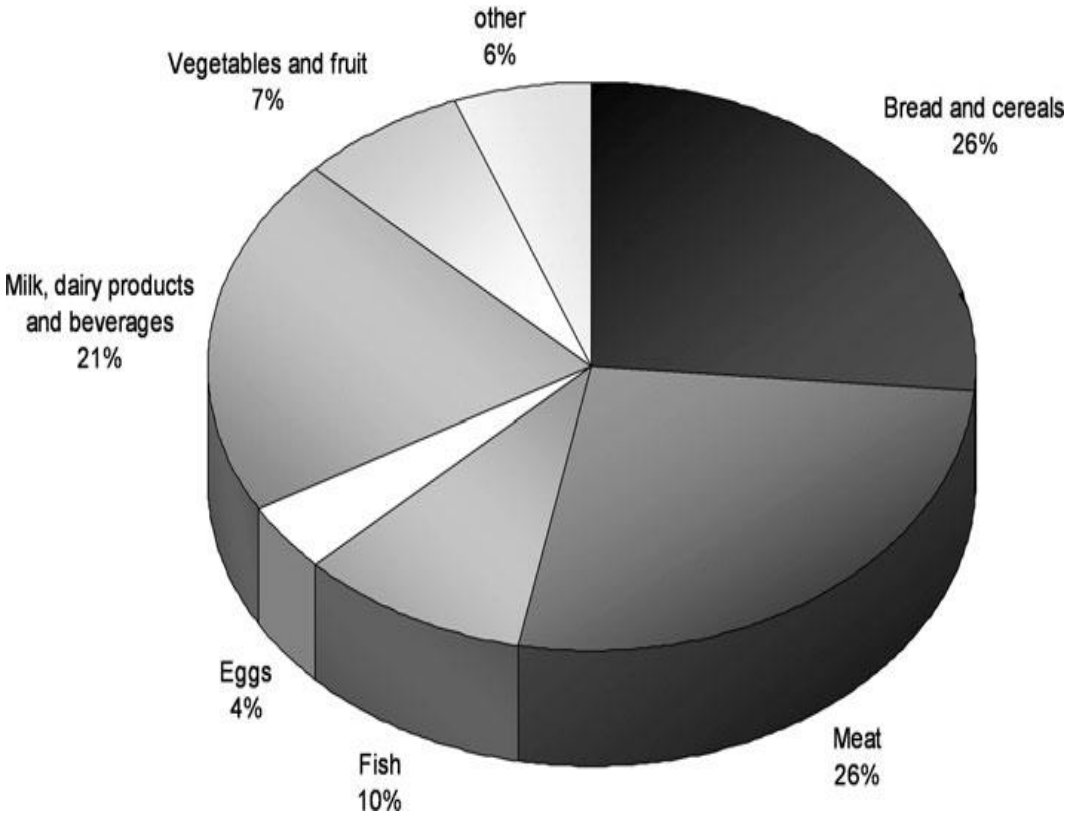

Figure 1.5 Uptake of selenium from different dietary sources adapted from (132)

1.11 Effect of arsenic exposure and selenium supplementation on oxidative stress and antioxidant levels

Arsenic exposure causes oxidative stress and damage of deoxyribonucleic acid (DNA)by the production of ROS(133). DNA damage can be evaluated by determining 8-hydroxy-2′-deoxyguanosine (8OHdG) level, a surrogate marker of oxidative stress or oxidative DNA damage. A remarkable relationship was found between the 8-OHdG concentration and As levels in urine(133).On the other hand, glutathione (GSH) is a thiol group-containing well-known tripeptide (cysteine, glycine, and glutamic acid) found in high levels in many tissues in the body. Glutathione plays essential role in decreasing oxidative stress,

maintaining redox balance, improving metabolic detoxification, and regulating the immune system (134). Total GSH concentration reflects the antioxidant status of the body. In mouse models, it has been shown that exposure to As decreased the levels of GSH (135). Glutathione is also used in various cellular processes, including cell differentiation, proliferation, and apoptosis. Thus, if GSH homeostasis is hindered, then it is associated with the etiology and/or progression of several human diseases, including cancer, diseases of aging, cystic fibrosis, and cardiovascular, inflammatory, immune, metabolic, and neurodegenerative diseases (134, 136, 137). Moreover, intervention with Se among congestive heart failure patients showed a remarkable elevation in plasma total GSH levels compared to placebo (138).

1.12 Impact of arsenic exposure and selenium supplementation on inflammation

Both experimental and epidemiological studies showed associations between As exposure and higher levels of various inflammatory markers, including acute-phase protein and pro-inflammatory cytokines (139-144). Chronic As exposure was found to be associated with asthma-like symptom and allergy (145). Whereas Se is not only essential in regulating immune functions (146); it also important for different non-specific immune response (147). Selenium deficiency is related to weaken the immune system (148).In inflammatory diseases, the reduction of Se content causes disturbance of the biosynthesis of selenoproteins (149). The combined supplementation with Se and vitamin E found to be beneficial to control allergic mediators as well as symptoms in rhinitis and asthma and reduce respiratory inflammation and airway mucus secretion, so that the obstructed bronchi remained open(150).

1.13 Biomarkers to assess inflammation

C-reactive protein (CRP) has been used as one of the most common inflammatory biomarkers (151). The level of CRP is elevated for numerous reasons including acute and chronic states, describing the etiology of infectious or non-infectious situations. However, high CRP levels are mostly related with infections(152). Additionally, the quantification of fractionated exhaled nitric oxide (FE_{NO}) has been used to diagnose specified airway inflammation.

In human, nitric oxide (NO) molecule plays it's biological activity, by regulating some processes including vascular and bronchial tone, inflammation, and neurotransmission (153). Nitric oxide exists in exhaled air (154) and can be measured by comparatively simple and reliable technique using chemoluminescence (155). The FE_{NO} mostly originates from the lower respiratory tract, mainly from the lung's airways, if nasal air is excluded(156, 157). Assessment of FE_{NO} candeliver important information regarding various inflammatory lung diseases such as asthma, avoiding the critical procedures associated with bronchial biopsy or sputum induction. Elevated concentration of FE_{NO} in subjects with asthma compared with control subjects (156, 158, 159)suggested the recommended use of FE_{NO} measurements in the diagnosis of asthma (160, 161).

1.14 Association of arsenic exposure with cardiovascular diseases

A number of studies demonstrated the associations between As exposure and the occurrence of hypertension and other cardio-metabolic diseases (21, 162-164). Experimental studies have also indicated the involvement of As exposure in the development of hypertension by promoting inflammation, oxidative stress, and endothelial dysfunction (21, 50, 165-167). In an animal model, As exposure was associated with

increased lipid oxidation in exposed rats compared to controls. The same study also showed that chronic As exposure caused altered rat lipidome, increased lipid oxidation, and dysregulated metabolic pathways, which usually linked with the chronic inflammation occurred in different diseases (i.e., keratosis, Bowen's disease, and kidney, liver, bladder, and lung cancer)(168). Another rat study showed detrimental effects of As exposure by reducing high-density lipoproteins (HDL)and HDL/LDL ratio, as well as increasing the levels of triglycerides (TG), total cholesterol, low-density lipoproteins (LDL), and electrolytes(169).

1.15 Effect of dietary selenium supplementation in regulating blood pressure and lipids

In biological system, Se performs it's roles mainly via Se-dependent glutathione peroxidases(GPx) and other seleno-proteins on redox signaling, antioxidant defense, immune response and in the regulation of thyroid hormone function (170-173).Intake of adequate level of Se found to be beneficial for CVD, cancer, and other chronic diseases(132, 174). Previous retrospective case–control studies demonstrated that, the blood Se concentrations of CVD patients were lower than those of the healthy population, indicating an inverse association between Se status and risk of coronary heart disease(175, 176).

1.16 Significance of dietary selenium supplementation through lentils

Because of the role of Se to antagonize the toxic effects of As, a number of supplementation trials with Se have been conducted in the treatment of chronic As poisoning (105, 108, 109, 121, 122). Due to the compliance issue, long duration intake of daily supplements in the form of pills found to be challenging, especially for younger

population, and there can be added expense. Thus dietary intervention could be feasible, sustainable, and protective.

To treat various chronic diseases, including diabetes, hyperlipidemia, hypertension, etc., people usually take recognized drugs. But for the poor people it is difficult to bear the cost of drugs if those need to be taken regularly, additionally most drugs have some side effects. Thus the use of complementary therapies and modification of the dietary patterns are recommended to improve the condition of different chronic diseases.

In Bangladesh, lentils are consumed in regular basis. Lentils are fall in the legumes class that has many beneficial effects for maintaining good health and give protection against metabolic diseases such as type 2 diabetes and CVDs (177). According to food ingredient table data, lentil seed contains higher levels of fiber (3.7 gr/100) and has a low glycemic index (21.1). After the germination of lentil seeds, the amount of fiber and protein is increased (178). It has been found that in diabetic patients, after consumption of lentils, total cholesterol and glucose levels significantly decreased (179), while consumption of lentil sprouts reduced glucose.

In lentils, Se is found in highly bioavailable form, L-selenomethionine (4, 5)which is quickly absorbed into the blood once ingested. People from Bangladesh and other South Asian countries, daily consume red lentils as lentil soup (locally known as dahl) and as other recipes. Though lentils are grown in Bangladesh, but still the annually harvested amount of local lentils could not fulfill the national requirement. Thus a large proportion needs to be imported to meet the annual national demand. Canada is known as the world's largest producer and exporter of lentils(180). Moreover, the soils where red lentils are

grown in western Canada are rich in Se with high and safe Se levels ranging from 425 to 1884 μg/kg(181), unlike most other production regions globally,.

Thus incorporating high-Se lentils into the daily meals of As-exposed Bangladeshi families may help to reduce the symptoms of long-term As poisoning in a cost-effective, culturally acceptable, and nutritionally beneficial way.

1.17 Hypothesis:

We hypothesized that the regular intake of high-Se lentils from Saskatchewan, Canada for the 6 months' period, would lower the body burden of As and decrease As toxicity in a chronically As-exposed Bangladeshi population compared to those consuming low-Se lentils.

1.18 Objectives

The overall aim was to perform a double-blinded, randomized, placebo controlled trial to determine whether incorporation of high-Se lentils into the daily diet, providing at least the RDA dose of Se (55 ug), could result in health benefits in an As-exposed Bangladeshi population.

1.18.1 Specific Objectives

Specific objectives were to determine whether daily consumption of RDA level of Se (55 μg/L) through lentils can:

- reduce body burden of As by higher excretion of As through urine-stool while lower deposition in hair
- increase blood Se levels
- decrease the incidence of morbidity outcome
- improve the body mass index (BMI)
- change the concentration of acute-phase proteins (CRP) as well as decrease lung inflammation measured using exhaled nitric oxide as an indirect indicator
- reduce oxidative DNA damage (8OHdG) and improve antioxidant status (GSH)
- maintain blood pressure and plasma lipids levels in As-exposed individuals

Chapter 2

MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Shahrasti, a rural area in Chandpur district located ~109 km South-East of Dhaka, Bangladesh. According to Bangladesh Population Housing Census 2011, the total area of Shahrasti is 15.65 sq.km with a total population of 28,278. Males constitute48.41% of the population, and females 50.69%. Shahrasti has an average literacy rate of 67.0% (7+years)(182). There are 9 wards, 17mahallahs and 176 villages (**Figure 2.1**) in Shahrasti paurashava. In these rural places, households(HHs) usually have their own tube wells in the yards and the people from those HHs use water from those specified tube-well for drinking and cooking purpose. In Shahrasti, tube-well water arsenic (As)concentrations exceed the national standard of 50µg/L very often. According to Chandpur Public Health Engineering Department, 98% of the shallow tube wells in Shahrasti Paurashava have been found to be affected with water containing As at harmful levels.

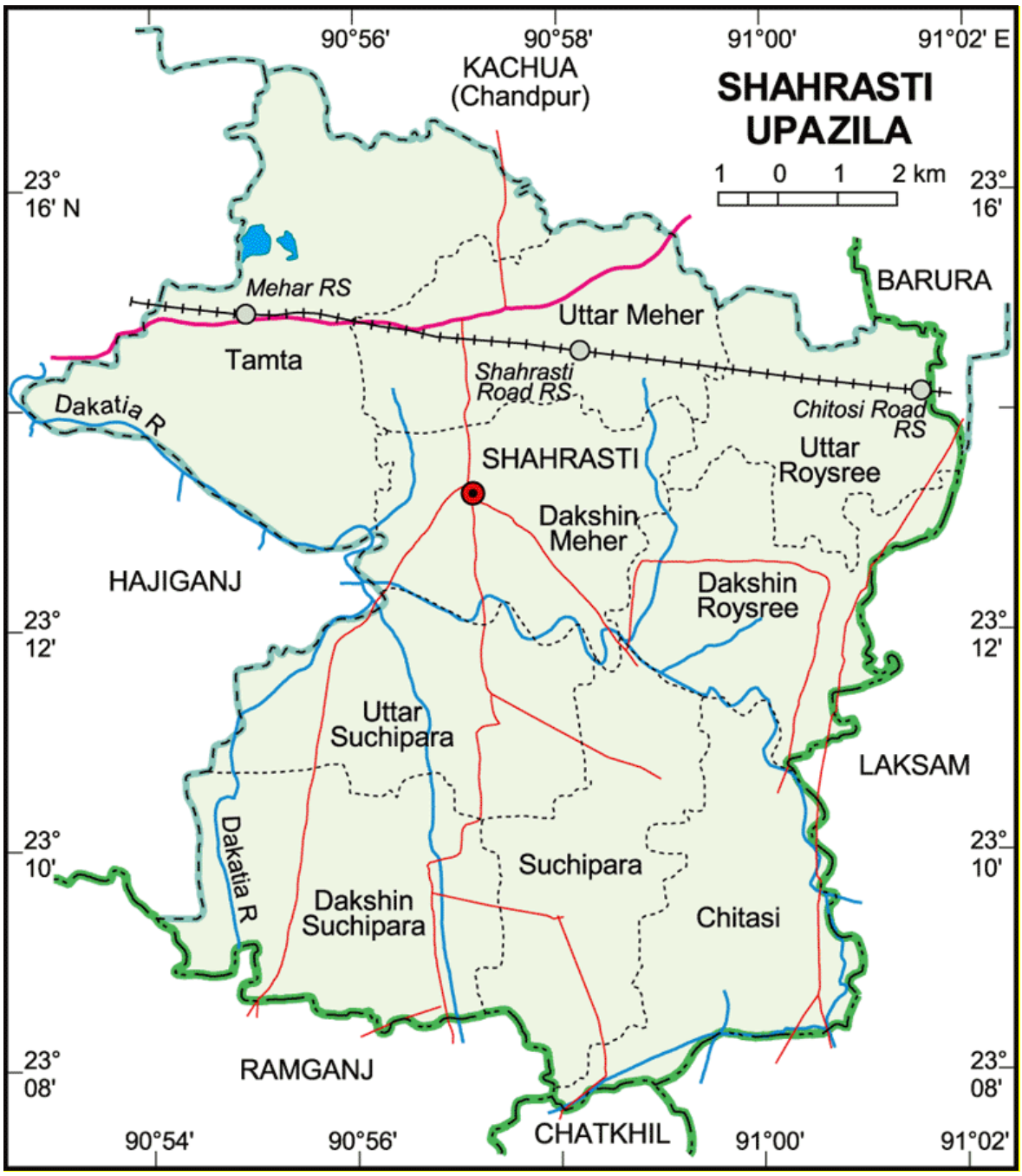

Figure 2.1 Map of Shahrasti Upazila of Chandpur District, adapted from (183)

2.2 Study design and participants

This study was a parallel, double-blinded, randomized placebo-controlled clinical trial over a period of 6 month (NCT0242992). The details of this clinical trial, including trial design, randomization, background details of the investigational product i.e. lentil, its production and nutritional analysis have been described earlier (184, 185). This community-based intervention trial was conducted among chronic As exposed healthy population of Shahrasti. During screening to include potential participants, tube well water As concentration was testes using a field kit (Hach EZ Arsenic test kit, Colorado, USA) that gave a semi-quantitative results of As.A total of 405 participants were randomly assigned to either Se-group (n=204) consuming high Se lentils or the control group (n=201) taking low Se lentils for six-month period. The participants were followed up to 6 months from baseline (**Figure 2.2**). The heath examinations and samples were collected at baseline, 3 and 6 months. The regular diets for the participants were not controlled for other sources of Se.

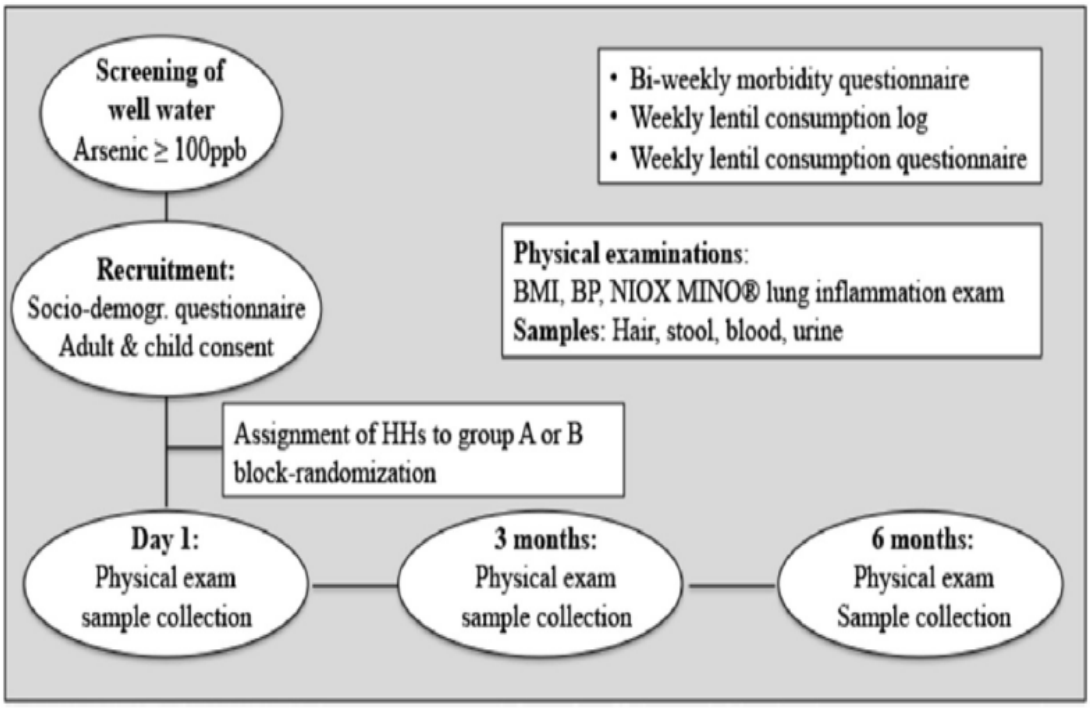

Figure 2.2 Flow diagram of the lentil supplementation trial; BMI, body mass index; BP, blood pressure; HHs, households(184)

2.2.1 Inclusion criteria

Eligible participants were:

- Aged ≥14 years.
- Consuming tube-well water containing more than100 µg/L of As.
- Apparently healthy having no chronic diseases.
- Currently not taking any Se pill.
- Having no possibility to migrate other places during the intervention period.

2.2.2 Exclusion criteria

Subjects who were

- Less than 14 years' age
- Consuming drinking water from tube well with < 100 µg/L of As.
- With history of chronic diseases including anemia, cardiovascular diseases, renal diseases etc.
- Pregnant and lactating women
- Possibility of migration during the intervention period within 6 months

2.2.3 Screening and enrolment of the participants

Initially we collected tube well water samples from 161 HHs for screening purpose by measuring As levels. From 161 HHs, 30 HHs were discarded due to having < 100 ppb (or µg/L) of As in their tube well water. Total 625 participants were screened from 131HHs with > 100 ppb of As. Among 625 participants, at first 181 were excluded for various reasons (i.e. 60 persons were having the chance to be migrated during the 6 months' study period, 30 persons were apparently unhealthy and 91 were not interested to be enrolled). During consenting additional 39 subjects refused to give their consents (**Appendix File 1 & 2**) to the study team. Finally, 405 participants from 94 HHs (47 HHs in each group) were enrolled, among them 201 were enrolled in control group and 204 in Se-group. From control group only one participant dropped out at month 6 while six participants dropped out from the Se-group at the same time point (**Figure 2.3**).

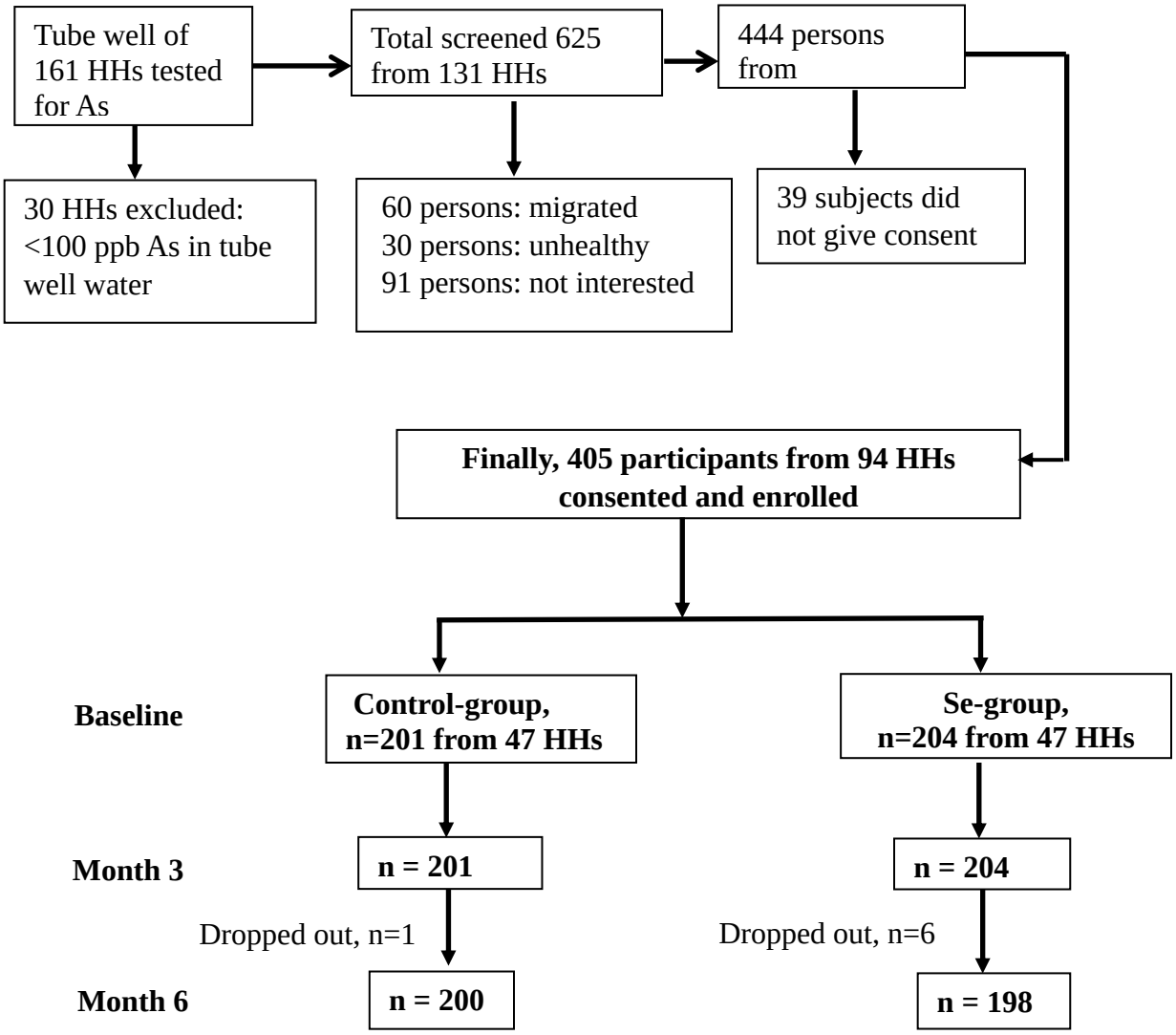

Figure 2.3 CONsolidated Standards of Reporting Trials (CONSORT) diagram showing screening, enrollment, allocation of dietary intervention, dropout and follow-up status of participants. Abbreviations, HHs=households; Se=selenium; As=arsenic.

2.3 Investigational product (Lentils)

Participants from Se-group received high-Se lentils while those from control group taken low-Se lentils. The high-Se containing red lentils were cultivated at Lucky Lake in Saskatchewan, Canada, where soils are naturally rich in Se (bio-fortification). Whereas

low-Se lentils were grown in Genesee, Idaho, a place in the northwestern USA, where Se content in soils is very low (181). The both types of lentils were similar in appearance. After harvesting all lentils were processed. Processing included de-hulling, polishing and packaging in either green or white colored 25-kg bags in Vanscoy, Saskatchewan. Samples from both types of lentils were tested for micronutrients, phytic acid, and macronutrients. The lentils were also analyzed for radiation to obtain the biosafety certification by the Saskatchewan Research Council, Saskatoon, confirming that the two lentil varieties were being suitable for human consumption. Blinding of lentils was occurred at the production site in Saskatchewan. The lentil type (either high or low-Se) was unveiled after the completion of trial and neither the study participants nor the study investigators knew about the lentil type until the analysis of urine, stool, hair and blood samples had been completed for As and Se measurement. The packaged lentils were transported to Bangladesh. After receiving in Chittagong port, Bangladesh, lentils were re-analyzed for radiation and phytotoxins, as a protective measure preventing the importation of contaminated grain in Bangladesh. Once the port permitted the lentils to release, all the lentil bags were transported to icddr,b store house, Dhaka for storage at room temperature (20-34°C).

2.3.1 Lentil Dose

The Se content in high-Se lentil varieties was 0.854 ppm (854 µg/Kg) and in low Se-lentil verities was 0.029 ppm. In intervention group (Se-group), each participant required to eat 65g of lentils daily to get the required daily allowance (RDA)of 55µg Se. Thus, participants of both arms received 65g of lentils per person per day for 6 months with the intervention arm receiving high-Se lentils (Se group), while control group

receiving low-Se lentils with a dose of 1.5µg Se/day. A scoop was provided to each enrolled HHs for measurement consistency (**Figure 2.4**). Participants were consumed the daily lentil dose in various cooked forms (i.e. Lentil soup, patties with smashed lentils etc.) (**Figure 2.5**). As earlier it has been shown that little or no loss of Se occurred as a result of ordinary cooking techniques i.e. boiling, baking, frying etc. (186). In our study, the participants mostly took lentils after cooking in different ordinary ways. Thus we do not expect any major loss of Se in high-Se lentils.

Figure 2.4 Scoop for measuring 65g of lentils per participant daily during the intervention period

Figure 2.5 Cooked lentils in various recipes

2.4 Randomization of participants

Screening of the participants was performed by measuring household water As range using field kit (Hach EZ Arsenic test kit, Loveland, CO). Eligible participants from an entire household (where family members taking meals from the same cooking pot) were assigned to either the control group consuming low-Se lentils or intervention group obtaining Se-rich lentil. For randomization the two groups were defined as A and B, either of which received Se-rich lentils. Participants from both groups were continued to take higher As containing drinking water during the intervention period even though the project field team repeatedly recommended the participants to use the nearest well with lower arsenic.

A block randomization method had been used to select the households. Once a family was recruited, eligible members of that family were assigned to either group A or B. The blocks were of variable length (containing 4-6 households), and both groups were randomized within each block. In rural Bangladesh, Bari is usually defined as a single plot of land where a number of households/families live while a family meant as a place where members taking meals from the same cooking vessel. In this trial each group contained equal numbers of households (47 household /group).

2.5 Data collection

2.5.1 Socio-demographic information

Socio-demographic information was collected once just after taking consent of the participants by the Field assistants (FAs). A structured questionnaire (**Appendix File 3**) was applied to collect detail information of family members (including name, gender, age, relationship with head of household, marital status, occupation and educational level),

information on socioeconomic status (SES) (including duration of living in the house, ownership of the house, building materials and average monthly income) and hygiene management information (i.e. use of hand washing soap at house, disposal of household garbage, sanitation, information of tube-well water for drinking and arsenic exposure related information). The SES score of the participants from a particular household was estimated from monthly expenditure of the family, the building materials including bricks, tin, straw etc.

2.5.2 Lentil intake compliance data

The families were received lentils in a weekly basis from the day of enrollment after the collection of baseline samples. After estimating the weekly required amount of lentils for particular families, the female village health workers (FVHW) supplied the lentils to the households. The female village health workers (FVHW) ensured the compliance of lentil consumption by visiting each household twice weekly and estimating the amount of leftover lentils in each household before the dispatch of next batch of lentils. To collect the information of lentil intake compliance another questionnaire was used (**Appendix File 4**). Any participant missing the daily meal due to travel outside the village or arrival of house guests for some days which affected the weekly stock of lentils was noted. The heads of the families informed about arrival of house guests and additional lentils were provided to these families with similar lentil dose as for study participants.

2.5.3 Anthropometric measurements

On the day of specimen collection, participants were invited to the field station for anthropometric measurements, bio-specimen collection and other examination. Anthropometric measures including weight, height and blood pressure were recorded at

baseline, month 3 and month 6 (**Figure 2.2**). Body weight was measured twice each time point using a digital Scale (TANITA HD – 318, Tanita Corporation, Japan) which was calibrated regularly with a standard weight of 20 kg. Height was measured by using a locally manufactured wooden stadiometer (with a precision to 0.1 cm). Blood pressure was measured twice using a blood pressure machine (ALPK2 Japanese ALPK2 V500) in sitting position after rest and relaxation for at least 15 min.

2.5.4 Morbidity information

Based on a set of structured questionnaires, morbidity information was collected by study field assistants (FAs)from the participants fortnightly (2-week recall data) **(Figure 2.2)** during scheduled home visits. The morbidity questionnaires included specific morbidity related questions concerning ever history of asthma, recent asthma incidences, skin problem, allergic skin diseases, respiratory illness (in terms of cold, cough, or difficult breathing), urinary tract infections (in terms of pain, burning, or difficulty during urination) with or without concomitant fever, diarrhea/loose stool including the duration of the morbidity symptoms (days of illness).

2.6 Specimen collection

All biological samples were collected from study participants in fasting condition at morning at field office. Peripheral (venous) blood, urine and stool (sub-sample) specimens were taken at baseline, 3 months (first follow-up visit) and six months (second follow-up visit) from start of the intervention. Hair was taken at baseline and at the second follow-up visit. Household waterof the participants were collected again after screening during the interventionperiod for obtaining more precise results of As. Fractionated

exhaled nitric oxide (FE_{NO}) was measured at baseline, month 3 and month 6 (**Figure 2.2**)(184, 185).

2.7 Assessment of arsenic exposure

2.7.1 Water arsenic assessment

Arsenic exposure was assessed by measuring total As concentration in household's tube well water at the icddr,b laboratory by using hydride generation atomic absorption spectrometry (HG-AAS) (AA-7000, Shimadzu, Kyoto, Japan) connected to an auto-sampler (ASC-6100, Shimadzu) and a hydride generation system (HVG-1, Shimadzu) (187).

Water samples (all household, n=94) were collected in 50 mL falcon tubes (BD, Franklin Lakes, NJ, USA), sent to Dhaka lab and stored at -20℃ freezer until analysis to measure water As concentration more accurately. On the day of analysis, water samples were thawed properly and 10 mL water was taken into a 15 mL falcon tube (BD, Franklin Lakes, NJ, USA), combined with 5M HCl (BDH, Poole, UK) and 20% potassium iodide (BDH) for reducing the pentavalent arsenic (As V) to trivalent arsenic (As III) by heating on a water bath at 80°C for 30 minutes. Blanks and standards were prepared in the same manner. Working standards for As were prepared daily from a stock As solution (1000 mg/L) (Sigma Aldrich, Steinheim, Germany).The detection limit was 1μg/L. Internal quality-control samples and standard reference materials for water (National Institute of Standards and Technology, USA)were used throughout the study to confirm accuracy and precision. Inter-assay variation for water was within 6.5%. Water-As is expressed in μg/L.

2.8 Analysis of biological samples for arsenic measurement

In biological systems, As is found as both inorganic and organic complex. Inside body As forms organic compound while combined with carbon and hydrogen. Thus biological samples (including urine, stool and hair) required acid digestion (BDH) inside a fume hood (ESCO FRONTIERTM, Singapore) before analysis.

2.8.1 Measurement of total arsenic in urine

Urinary total As (U-As) was measured by using hydride generation atomic absorption spectrometry (HG-AAS) (AA-7000, reconstitute the digested urine sample Shimadzu, Kyoto, Japan) connected to an auto-sampler (ASC-6100, Shimadzu) and a hydride generation system (HVG-1, Shimadzu). Urine samples were collected in pre-labeled plastic urine container (68). Before analysis, urine sample (0.5 mL) was taken into a pyrex glass tube (Corning, NY, USA), treated with concentrated nitric acid (69%) and heated at 150°C for complete digestion into a clear solution. The sample was dried to approximately 100 µL of solution for analysis. De-ionized water was added to and treated similarly as water samples to reduce As V to As III. Internal quality-control samples and standard reference materials for urine (UTAK Laboratories, Valencia CA, USA) were also digested similarly as urine samples and used throughout the study to confirm accuracy and precision. De-ionized water was added to reconstitute the digested urine sample. After digestion urinary total As (U-As) was measured by (HG-AAS)similarly as water samples(185).

Specific gravity of urine samples was measured using a digital refractometer (RD712 Clinical Refractometer; EUROMEX, Arnhem, the Netherlands) before taken for

digestion. The average specific gravity of an individual time point was used for adjusting urinary-As (U-As) concentration to compensate the variation in dilution (188).The detection limit for U-As was1 μg/L. Inter-assay variation for urine As analyses was within 9.8%. Urinary-As is expressed in μg/L.

2.8.2 Measurement of arsenic concentration in stool

After weighing, 0.5 g stool sample was taken into a pyrex tube and 2mL of concentrated nitric acid and 0.5mL of 70% perchloric acid (BDH) were added and kept at room temperature overnight. The acidified stool suspension was heated at 100ºC on hotplate for digestion and the clear solution was further dried at 180°Cto a volume of about 100 μL for further analysis. De-ionized water was added to the digested stool samples to make the final volume 10 ml. Digested stool samples were treated similarly as water samples to reduce As V to As III. Stool total As content (stool-As) was measured using HG-AAS(185). The detection limit for stool-As was 1μg/L. Inter assay variation for stool As analysis was within 10.3%.

2.8.3 Measurement of arsenic content in hair

Hair samples were cut near to the skull from the nape of the neck to quantify As diposition in hair (hair-As). Hair was washed, snipped, digested in 70% nitric acid using an Ethos EZ microwave digester(Milestones, Shelton, CT, USA) and tested at the University of Saskatchewan following the standard IAEA protocol (189). Digested hair samples were evaporated and re-suspended in HCl for measurement by HG-AAS. Reference material for hair-As analysis (ERM®-DB001) was obtained from the European Commission, digested and analyzed with every batch of samples (every 27 samples), to validate the constancy of the results(185). Hair-As expressed as μg/kg.

2.9 Measurement of urinary selenium

The concentration of total Se in urine (U-Se) was measured for a subset ofsamples (n=232) at baseline, month 3 and month 6 using iCAP Qc ICP-MS (Thermo Scientific)atthe University of Saskatchewan.Inductively coupled plasma mass spectrometry (ICP–MS) is an instrumental analytical technique based on the use of a high temperature ionisation source (ICP) that converts the atoms of the targeted elements in the sample to ions while mass spectrometer further quantifies the elements on the basis of their molecular weights and chemical structures.Beforeanalysis in the ICP-MS for measuring total Se levels, urine samples werediluted with 2.25% nitric acid and 0.5% hydrochloric acid.Working standards were prepareddaily from a standard Se solution (1000 mg/L) (Ricca ChemicalCompany, Arlington, USA).The Se analysis was done in kinetic energydiscrimination cell mode using hydrogen gas. Internal controls (CDC Redberry lentils from Saskatchewan,calibrated against NIST standards i.e NIST 1568b rice flour and NIST 1573a tomato leaves) with known Se concentrations were used to validate the constancy of the results of U-Se. Sample recovery rate for Se was approximately 105%.

2.10 Measurement of selenium and arsenic in erythrocytes

Peripheral blood was collected from the study participants in fasting condition in trace element free lithium-heparinized coated beads containing tubes (S-monovette,SARSTED, Nümbrecht, Germany). Heparinized whole blood was placed on the top of ficoll-Paque PLUS (GE Healthcare, Chicago, Illinois, United States) density gradient media; plasma and blood cells were separated by density gradient centrifugation. Plasma and erythrocytes samples were aliquoted and stored at −80 °C.

A sub-sample of erythrocytes(n=194) were taken for analysis at baseline and month 6. The samples were treated with 0.5% nitric acidfor Se and As analyses using ICP-MS (iCAP Q ICP-MS, Thermo Fisher Scientific, Waltham, MA) at the University of Saskatchewan(185). Internal controls (CDC Red berry lentils from Saskatchewan, calibrated against NIST standards i.e NIST 1568b rice flour and NIST 1573a tomato leaves) with known Se concentrations were used to validate the results of Ery-Se. Sample recovery rate for Se was approximately 105%.Ery-Se. Total Se(Ery-Se) and As (Ery-As) levels were reported as μg/L in packed red blood cells (erythrocytes).

2.11 Measurement of erythrocyte total glutathione (tGSH) concentration

Glutathion is a tri-peptide thiol (γ-glutamylcysteinyl glycine) antioxidant which exists in two forms within the cell; reduced sulfhydryl (GSH) and the oxidized glutathione disulfide (GSSG) form. Intracellular concentration of total glutathione (tGSH) indicates oxidative stress. In this study tGSH (considering both GSH and GSSG) was measured by recycling assay method- where 5,5'-dithio-bis (2-nitrobenzoic acid)(DTNB)(Sigma Aldrich, Steinheim, Germany) was used. The assay was also dependent on the specificity of glutathione reductase (GR) (Sigma) enzyme. In packed erythrocyte samples tGSH was measured by reducing the GSSG into GSH during the assay.

Before analysis for measuring total glutathione level, blood sample was processed and de-proteinized. One mL heparinized blood was taken in a pre-labeled eppendrof. Plasma was separated by centrifugation at 2500g 4^{o}C for 5min and removed. The erythrocytes were washed twice with 4 volume of isotonic phosphate buffer (pH7.4). The washed erythrocyte pellet (125μL) was lysed with 4 volumes de-ionized water. Erythrocyte lysate was mixed with equal volume of 10% metaphosphoric acid (MPA)

(Sigma) in stock buffer, KPE (0.1M potassium phosphate buffer with 5mM EDTA disodium salt, pH7.5) mixed thoroughly by vortexing, kept at 0-4^{o}C for 2min and centrifuged at 3000g at 4^{o}C for 10min. The per, clear supernatant was collected carefully for tGSH measurement as described earlier (190). Briefly, the assay was based on the reaction of GSH with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (also known as Ellman's reagent). Reaction produced chromophore 2-nitro-5-thiobenzoate anion (TNB) that had maximal absorbance at 412nm, and oxidized glutathione (GS-TNB) adduct. The rate of TNB production was proportional to GSH level in the sample. The GS-TNB adduct was reduced by GR to 2GSH using NADPH as co-factor. Therefore, [tGSH] = [GSH]+ 2[GSSG]. Rate of absorbance(ΔA) at 412nm/min was constructed to be linear for consistency of measurement, and was linearly proportional to the total concentration of GSH. The concentration of tGSH was expressed as µM.

2.12 Measurement of plasma 8 OHdG concentration

Plasma concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were analyzed by a competitive immunoassay using HT 8-oxo-dG ELISA Kit II (Trevigen, Inc. Helgerman Ct. Gaithersburg, MD) according to the manufacturer's instruction. Before analysis, the samples were centrifuged at 2500g for 15 minutes at room temperature to remove the interfering proteins, which could interfere with the measurements of 8-OHdG. The low limit of detection for 8-OHdG was2 nM. Laboratory prepared internal plasma pool sample was used to validate the constancy of the results. The overall intra and inter-assay coefficient of variation was 7.2 and 12.3% respectively (which is within the recommended CV%).

2.13 Assessment of fractionated nitric oxide (FE_{NO}) in exhaled air

The Fractional Exhaled Nitric Oxide (FE_{NO}) concentration was measured by using a portable handheld electrochemical device (Niox Mino, Aerocrine, Solna, Sweden).There is an electrochemical sensor present in each of the device. That electrochemical sensor was pre-calibrated by the manufacturer for the programmed tests for 100analyses.All the analyses were performed in the morning time (7-9a.m) to avoid the variation day time as the level of FE_{NO} differs for different times on a day (i.e. morning, afternoon, evening and night). Following the instructions of the American Thoracic Society/European Respiratory Society Standardization guidelines (American Thoracic and European Respiratory) the procedures of FE_{NO} test were performed. Each participant was requested to vacant their lungs by expiration and then take a deep breath of air that is free of NO through the one time use mouthpiece filter, up to the lung capacity. After that, participant asked to exhaled the air through the filter at an exhalation pressure of 10–20 cmH_2O to keep a constant exhalation rate of 50 ± 5 ml/s for 10 seconds (**Figure 2.6**). The pre-calibrated electrochemical sensor gives readings in parts per billion (ppb). If participant failed to follow the total procedure and could not complete the analysis successfully with one attempt, he/she was being tried for three repeated tests with 10 min rest between each test. There is an instruction from the manufacturer (Niox Mino) which was followed periodically to replace the sensor in the device.

Figure 2.6 NIOX Mino device for measuring nitric oxide in exhaled air (FE_{NO})

2.14 Measurement of plasma lipids and C-reactive protein

Aliquots of frozen plasma samples were thawed, mixed by vortexing and analyzed in auto-analyzers as indicated by the manufacturer.

Cholesterol (Chol) and triglyceride (TG) concentrations were measured by enzymatic colorimetric method on fully automated Clinical Chemistry analyzer Cobas c311 (Roche Diagnostics Mannheim, Germany) using Roche kits. The method for Chol analysis was standardized according to Abell/Kendall and also by isotope dilution/mass spectrometry. This complies with the requirements of the National Institute of Standards and Technology (NIST). The method for TG was standardized against the isotope dilution/mass spectrometry (ID/MS) method. High density lipoprotein (HDL) and low density lipoprotein (LDL) were measured by homogeneous enzymatic colorimetric method on Cobas c311 using Roche kits. The method for HDL was standardized against the designated CDC reference method. This standardization met the requirements of the "HDL Cholesterol Method Evaluation Protocol for Manufacturers" of the US National

Reference System for Cholesterol, CRMLN (Cholesterol Reference Method Laboratory Network), November 1994. The method for LDL was standardized against the beta quantification method as defined in the recommendations in the LDL Cholesterol Method Certification Protocol for Manufacturers. To calibrate the instrument, Calibrator for automated system (C.f.a.s.) HDL/ LDL-C plus was used for HDL and LDL and c.f.a.s.U (Roche diagnostics) was used for Chol and TG. The inter assay coefficient of variation for plasma TG, Chol, HDL and LDL were <5%. Plasma lipid levels were reported as mg/dL.

Plasma concentration of high sensitive C-reactive protein (hsCRP) was measured as a marker of recent or current infection in the participants with Roche automated clinical chemistry analyzer (Cobas c311, Roche Diagnostics, Mannheim, Germany) using a commercial kit [Tina-quant CRP (hs), Roche Diagnostic GmbH]. Commercially available QC materials from Roche Diagnostics were used for quality control purposes. The inter assay coefficient of variation was 2.8%. Plasma hs-CRP was expressed as mg/L.

2.15 Power estimation and statistical analysis

Sample size calculation was performed on the based on a clinical trial in Mongolia (105). In that trial, after 9 months of intervention, hair-As concentration of the As exposed participants was decreased by 10% in Se-supplemented (100μg Se in yeast/day) group compared to that of placebo group. By considering 10% decrease in hair-As, with a power of 80%, a type-1 error of 0.05 and a 25% attrition rate comes to 190, rounded up to 200 participants per group (**Table 2.1**). However, as we enrolled all the apparently healthy subjects aged ≥ 14 years from an entire family, the number of participants in each group was slightly different.

Table 2.1: **Sample size calculation**

Tail	Expected difference in mean (d)	SD	β	Power(1-ß)	Sample size/group	Total sample size with lost to follow up (10%)	Proposed sample size/group
2-tailed	0.1 µg/g	0.1-0.15	.05	80	152	167	200

Statistical analyses has been conducted using the software PASW 22.0 (SPSS Inc. Chicago, USA), Stata/IC (version 13) (StataCorp, Texas, USA)and figures were prepared by using GraphPad Prism (version 7.05). Data distribution patterns were checked using scatter plots; normality and homogeneity of variances were formally evaluated by descriptive statistics. Primary analyses were performed by intention-to-treat population and the secondary analyses were performed by per-protocol. Continuous variables were analyzed for normality with the QQ-normalplot. When necessary, continuous outcome variables were ln (natural logarithm) transformed to obtain normally distributed residuals with a homogeneous variance.

The magnitude of the association between outcomes (urine, stool and hair As,As metabolites and proportion of As metabolites, Ery-Se, Ery-As, U-Se, plasma lipids, plasma tGSH, plasma 8OHdG, FE_{NO}, BMI etc.) and exposure (intervention groups) was observed by performing different types of statistical approaches. Two-way analysis of variance (ANOVA) test and multivariable adjusted regression analysis were performed to determine the changes in the levels of different outcome variables between the 2 intervention groups (control and Se-group) at baseline, month 3 and 6. Within each dietary group, the estimated mean change between baseline and follow-ups (months 3 and 6) of outcomes were analyzed using repeated-measures (RM) ANOVA.

To further assess the overall changes in urinary and stool-As excretion from baseline to month 6 between groups, generalized estimating equation model (GEE) was applied. Models were adjusted with covariates (age, sex, BMI, water As, and household expenditures at baseline) that affected the model R^2 by 5% or more in the best fitted regression model. The association between different outcome measures were assessed by multivariable adjusted regression analysis. For binary outcomes of morbidity, a multilevel mixed effect logistics regression model was used to evaluate the odds ratio (OR) of morbidity in the Se vs. control group.

2.16 Ethical consideration

Informed consent was obtained from all adults (>18 years) and when a child participant (between 14–18years) recruited, assent was collected (**Appendix file 1 and 2**). All forms had been translated into Bengali. The study team explained the trial procedures to the participants in details and addressed queries of the participants if there were any. Enrolled participants were informed that they could refrain from the study at any time point without affecting their access to routine health services. This study was conducted with approval by the Ethics Review Committee, of icddr,b (Protocol No. PR-14013) and Institutional Review Committee of University of Dhaka. A Data and Safety Monitoring Board (DSMB) was formed at icddr,b for monitoring the safety of the study participants and data.

Chapter 3

RESULTS

The results of the study to assess the impact of eating high-Se containing lentils into the everyday diet for mitigating As-related health problems in rural Bangladeshi population are presented in this chapter. The study was conducted on Arsenic (As)-exposed families with 204 subjects consuming bio-fortified high-Se lentils (Se-group) and 201 subjects consuming low-Se lentils (control group). In this study, we primarily aimed to examine whether RDA level of Se supplementation through naturally bio-fortified lentil could ensure health benefits to chronically high level of As exposed population by increasing the excretion of As from the body and body mass index (BMI) as well as by reducing frequency of morbidity outcomes. Our secondary aims were to determine whether the dietary supplementation has any impact on the body's antioxidant defense (demonstrated by the concentrations of oxidative stress marker and antioxidants), on the levels of inflammatory markers, blood pressure and plasma lipids.

3.1 Baseline socio demographic characteristics of the study participants

Of the 605-screened subjects, 405 participants were enrolled in the study; among them, 201 persons were enrolled in the control group, while 204 were in Se-group (Figure 2.3). The mean age of the study participants was 33.9 years, with the range from 14-75 years. The majority of the participants were females (59.26%). Among all participants, men were significantly older than women (mean age 35.44 and 32.79 years for men and women respectively) at the time of enrolment. The mean age of Se-group participants (32 years) was significantly higher than that of control group (35.77 years) ($p=0.017$) and in control group more females (n=129) were recruited than in Se-group (n=111) ($p=0.045$).

The mean age of the participants between the two groups was comparable. We categorized the participants by age (≤20, 21-45, and ≥46 years) and found no significant difference in number of different aged participants between the two groups. At baseline, female participants had higher BMI than males ($p<0.001$), while no significant difference was obtained in BMI level between the participants of control and Se- group ($p=0.72$). Though we recruited participants from the screened households for those the tube well water-As levels were >100 μg/L, however, participants from Se-group found to have higher levels of As($p=0.083$) in drinking water from tube well (Table 3.1). Thus during statistical analysis age, sex, BMI at baseline, water As levels were used as covariates.

Table 3.1 Baseline characteristics of the study participants.

Variables		Control group (n=201)	Se group (n=204)	p-values
Age, years		35.8±1.2	32.0±1.0	0.02
	≤20 years	48 (23.9)	64 (31.3)	
	21-45 years	98 (48.6)	93 (45.6)	0.22
	≥46 years	55 (27.4)	47 (23.0)	
Sex, number (%)				
	Male	72 (35.8)	93 (45.6)	0.04
	Female	129 (64.2)	111 (54.4)	
BMI (kg/m^2)		21.8±4.1	21.6±4.2	0.70
	Male	20.7±3.4	20.7±3.5	0.96
	Female	22.4±4.3	22.4±4.6	0.96
Water arsenic, µg/L		442.3±125.3	466.2±150.7	0.08
Education status, number(%)				
	Uneducated	39 (19.4%)	24 (11.8%)	
	Primary level	46 (22.9%)	53 (26.0%)	0.17
	Secondary level	94 (47.8%)	107 (52.5%)	
	Graduate	22 (10.9%)	20 (9.8%)	
Occupation, number(%)				
	Unemployed	12 (6.0%)	11 (5.4%)	
	Housewife	96 (47.8%)	74 (36.3%)	
	Student	46 (22.9%)	58 (28.4%)	0.12
	Service	7 (3.5%)	5 (2.5%)	
	Business	40 (19.9)	56 (27.5%)	
Household expenditure (Tk/month)		17099±4378	17745±5308	0.18

Data are presented either as mean ± standard deviation or number with percent in parenthesis. P-values are estimated by independent sample t-test or chi-square test. BMI, Body mass index. TK, Taka i.e., the currency of Bangladesh.

Only 10.3% of study participants completed their graduation while 49% passed the secondary level of education. A substantial number of participants (41.97%) were

homemakers and 26.67% involved in either service or business (Table 3.1). The socio-economic status of the participants of the study groups did not differ. Household expenditure was similar for the participants of both groups. Household expenditure also considered as one of the covariates.

3.2 Nutritional assessment of lentils

After cultivation and harvesting, samples of the lentils were analyzed for macronutrients, phytic acid, and essential minerals. Except for Se, the two lentil varieties were nutritionally similar in terms of macro- and micronutrients and the anti-nutrient, phytic acid content (**Table 3.2**). The As content in both types of lentils was very low (<0.001 ppm).

Table 3.2. Macronutrient and micronutrient content of the two lentil types**

Elements	High Se lentils	Low Se lentils
Macronutrients (% by weight)		
Protein	**26.22**	**27.73**
Starch	**38.00**	**37.00**
TDF	**8.48**	**6.66**
Fat	**0.78**	**0.77**
Ash	**2.77**	**3.16**
Phytochemical (g/kg)		
Phytic acid	**0.61**	**0.72**
Minerals (mg/kg)		
Calcium	**327.88**	**377.51**
Potassium	**10.45**	**10.94**
Sodium	**71.74**	**75.00**
Magnesium	**786.30**	**943.00**
Copper	**9.34**	**11.44**
Iron	**75.75**	**65.30**
Zinc	**42.15**	**51.90**
Manganese	**16.95**	**14.61**
Selenium	**0.85**	**0.03**
Arsenic	**<0.001**	**<0.001**

**Taken from Krohn et al, 2016 (184). TDF, total digestible fiber

At the end of the study, the left-over study lentils were analyzed for the measurement of micronutrients. Table 3.3 describes that, similar concentrations of these nutrients in both high- and low-Se lentils at the end of the study approving the stability of the nutrients, including Se.

Table 3.3 Micronutrient contents of lentils at the end of the study

Lentil type	Micronutrients	RDA (adult men) mg/day	In 65g of lentils mg	% RDA from 65g lentils
	Iron (mg/kg)			
Low-Se (control group)	57.07±1.35	20.5	3.71	18.09
High-Se (Se-group)	68.59±3.68		4.46	21.75
	Zinc (mg/kg)			
Low-Se (control group)	48.77±1.98	11	3.17	28.82
High-Se (Se-group)	40.96±1.28		2.66	24.21
	Magnesium(mg/kg)			
Low-Se (control group)	965.06±15.62	420	62.73	14.94
High-Se (Se-group)	836.38±24.56		54.36	12.94
	Folate (µg/100g)			
Low-Se (control group)	201.99±3.16	400	131.29	32.82
High-Se (Se-group)	172.65±4.82		112.22	28.06
	Selenium (mg/kg)			
Low-Se (control group)	0.03±0.01	0.05	0.002	3.13
High-Se (Se-group)	0.99±0.07		0.06	116.76

Data are presented as mean±SE. The table includes the recommended dietary allowance (RDA) of micronutrients based on eating 65g lentils per day for adult men for simplicity (RDA differs for pregnant and non-pregnant women for most micronutrients and vitamins).

Both types of lentils (high-Se and low-Se content lentils) provided the same levels of nutrition except for Se to the study participants and the levels of micronutrients in the two varieties of lentils were stable since production till the end of the study period.

3.3 Arsenic levels in drinking water

In the study area (rural community of Shahrasti), we tested well water from 161 households' tube wells for measuring As concentration. We found only 7.4% tube well's water contained As levels within the WHO recommended range (<10 µg/L), 9.4 % tube

wells had As level below the national recommendation of As (<50 µg/L) and more than 80% (80.3%)tube well water contained relatively high levels of As (>100 µg/L). In this study, we enrolled participants from the 94 HHs from initially 161 screened HHs, where tube well water-As levels were >100 µg/L. Among the enrolled HHs, majority of the tube wells (63.7%) had water As level > 250 µg/L while a considerable portion of tube wells (32.3%) contaminated a very high level of As (>500 µg/L). For both study groups, the similar types of water As distribution pattern was noticed (**Figure 3.1**).

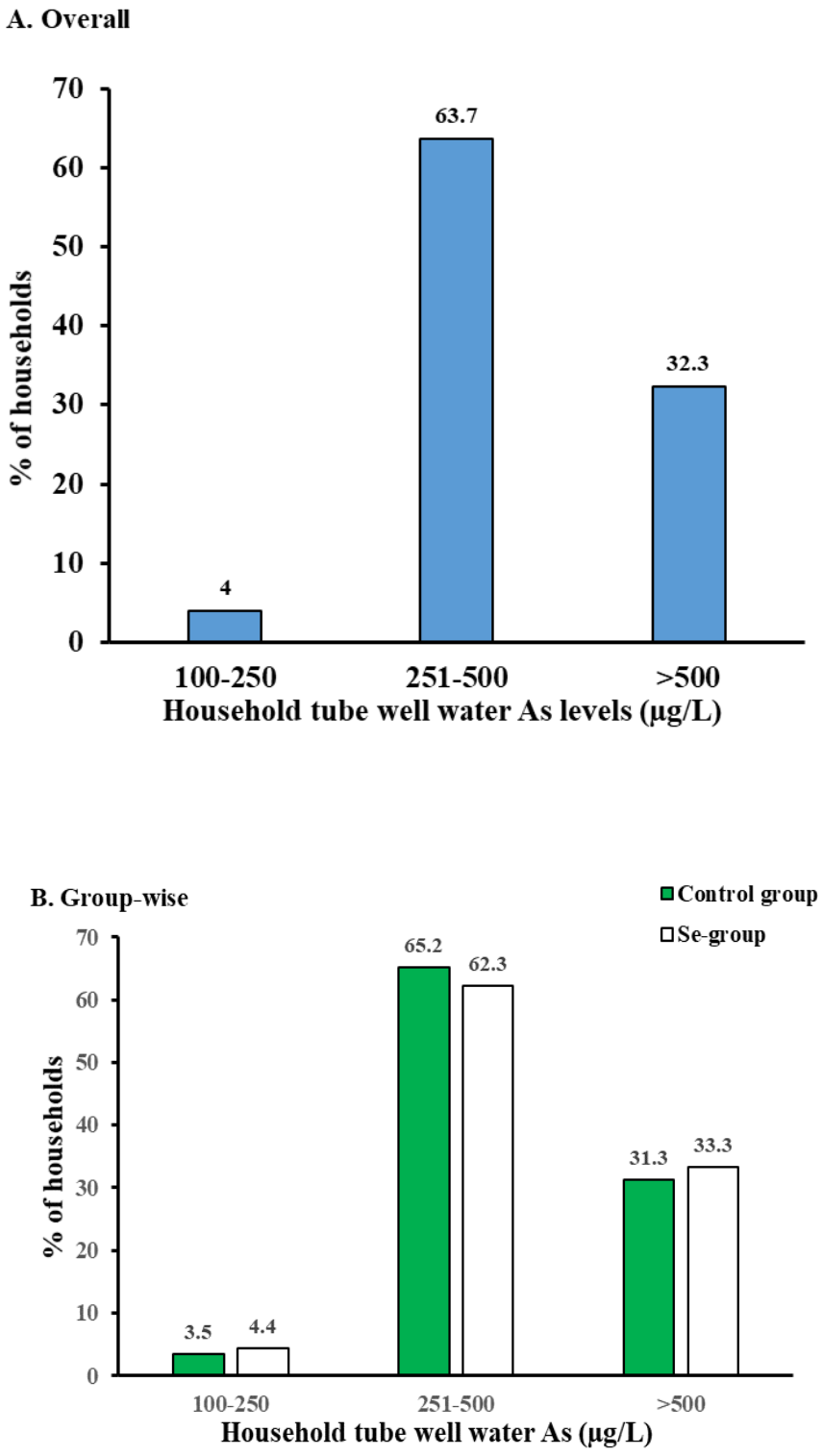

Figure 3.1 Distribution of tube well water As levels among screened households. A. Tube well water As in all households, B. Tube well water-As in control and Se-group.

In our study area, Shahrasti, Matlab, in the district of Comilla, Bangladesh, >80% of tube wells were contaminated with very high levels of As.

3.4 Lentil intake compliance

We have collected information on lentil intake compliance by measuring the left over lentil content from the weekly supplied lentil bulk as well as by using structured questionnaire collected twice in a week, i.e. how many times in a day any participants had taken the cooked lentils. In the first three months, >90% participants (for control group 96% and for Se-group 97.5%) took required amount of lentil on a regular basis. Whereas even with extensive monitoring by female village health workers, in the last three months of supplementation, the lentil intake compliance dropped significantly to ~80% for both the control (79.6%, $p<0.001$) and Se-group (82%, $p<0.001$) compared to first 3 months of the trial.

3.5 Concentration of arsenic in various biological samples

Total As concentration was measured in urine, stool, hair, and erythrocyte samples (sub-samples) at different time points. Arsenic levels in urine, stool and blood describe the recent exposure while hair As concentration reveals longer exposure. The measurement of total urinary As is usually used as the most common biomarker for iAs exposure (191).

3.5.1 Excretion of arsenic via urine

In this study, U-As concentrations at different time points (at baseline, midpoint and end point) were analyzed for evaluating the excretion levels of As from the body, which further reflects the current body load of As.

The comparison of U-As at different time points has been evaluated by two-way ANOVA and multivariate-adjusted regression analysis (**Table 3.4**). At baseline, the U-As levels of the participants between the two groups were similar. At month 3, a significant increase in U-As excretion was demonstrated in the Se-group(p=0.049) compared to control. Whereas at month 6, a non-significant increased excretion of U-As was found in Se- group (p=0.115) which may due to the decreased lentil intake compliance of the participants during the last three months of supplementation (**Table 3.4**).

Table 3.4 Changes in urinary arsenic (U-As) concentrations (specific gravity adjusted) between control and Se-group[a]

Variable	Control (n=201)	Se-Group (n=204)	β (95% CI)	p-value
At baseline				
U-As (µg/L) [a]	205.7±10.8	205.3±10.8	-0.02 (-0.08, 0.05)	0.98
At month 3				
U-As (µg/L) [a]	211.8±10.8	240.5±10.9	28.5(0.001, 58.7)	**0.049**
At month 6				
U-As (µg/L)[a]	193.7±10.3	216.9± 10.3	20.4(-8.76, 49.6)	0.11

Data are presented as mean±SE; [a]Data are log-transformed for statistical analysis. AAS, atomic absorption spectrophotometry2-way ANOVA is used to estimate the p-values. Multivariable adjusted regression model analysis is performed to calculate β-coefficient and 95% CI. β-values are considered in Se-group compared to control. The model is adjusted by age, sex, BMI at baseline, tube well water arsenic, and household expenditure.

Repeated measure analysis showed a significant increase in U-As excretion levels at month 3 compared to baseline (p=0.001) but not at month 6 in the Se-group (p=0.264). The excretion levels of U-As in the control group between time points were unchanged (at month 3, p=0.342 and at month 6, p=0.203 compared to baseline).

Longitudinal analysis by generalized estimated equation (GEE) method considering the U-As excretion in all the time points exhibited overall higher As excretion

in urine in the-Se-group compared to the control group over time (p=0.009), particularly in females(p=0.002) (**Table 3.5**).

Table 3.5 Overall changes of urinary arsenic concentrations (U-As) in the Se group compared to the control during 6 months' intervention with lentils

Variable	Participants	β[a, b] (95% CI)	p-value
U-As*	All	0.30(0.07, 0.52)	**0.009**
	Male	0.16(-0.18, 0.49)	0.36
	Female	0.39(0.09, 0.68)	**0.011**

* U-As concentrations are adjusted with specific gravity. Control group is considered as a reference; β, regression coefficient; CI, confidence interval. [a] Model is adjusted by age, sex (when all), BMI at baseline, tube well water arsenic, and household expenditure and time. [b]Overall changes are obtained considering baseline, month 3 and month 6 follow-up visits. p-values are calculated using longitudinal repeated-measure analysis (generalized estimating equation model, GEE).

3.5.2 Excretion of arsenic through stool

Stool As concentration is also expected to reflect the current or recent exposure similar to urine. **Table 3.6** describes the changes of stool-As concentrations for a subsample(n=132) of the participants. In contrast with U-As, no significant changes were obtained in stool-As excretion at any of the time points between the participants of Se- and control group.

Table 3.6 Changes in Stool-As concentration in Se- group compared to the control group at different time points

Stool-As (µg/L)	Control (n= 61)	Se-Group (n= 71)	β (95% CI)	p-value
At baseline	234.4±1.1	251.2±1.1	42.7(-24.8, 110.2)	0.53
At month 3	239.8±1.1	263.6±1.1	39.0(-25.6, 103.6)	0.38
At month 6	271.0±1.1	241.5±1.1	-24.3(-96.9, 48.3)	0.33

Values are shown as mean±SE; 2-way ANOVA is used to estimate the p-values. Multivariable adjusted regression model analysis is performed to calculate β-coefficient and 95% CI (confidence interval). β values are considered in Se-group compared to control. Model is adjusted by age, sex, BMI at baseline, tube well water arsenic, and household expenditure.

Repeated measure analysis was performed considering those subjects for whom stool samples were available for all the three-time points. No significant changes were noted in stool-As concentration at month 3(p= 0.82) and at month 6 (p=0.32) compared to baseline in control group(n=59). Similarly, in Se-group (n=66), the stool-As level remained unchanged at month 3 (p= 0.51) and month 6 (p=0.91) compared to baseline (Data not shown). GEE analytical model also showed no changes (β=0.02; 95% CI=-0.24, 0.28; p=0.88) in the stool-As levels in the participants between the two groups over the 6 months' intervention period. When participants of the two groups were categorized by sex, separately for male (β=-0.03; 95% CI=-0.45, 0.40; p=0.91) and female (β =0.08; 95% CI=-0.23, 0.41; p=0.59) also no significant changes in stool-As concentration were noted between Se- and control group during the intervention period.

3.5.3 Deposition of arsenic in hair

Hair-As concentration has been measured for all the participants at baseline and month 6. Hair might be considered as an excretory pathway, and once incorporated in the hair, the As is not biologically available.

Both two-way ANOVA and multivariable-adjusted regression analysis showed no significant difference in hair-As concentration between the two groups (β=−0.017; 95%CI=−0.07, 0.032; p=0.49) after 6 months' intervention while within each group between three time points the hair-As levels also remained unchanged (data not shown). However, when stratified by sex, female participants in the control group had notably greater accumulation of As in the hair at month 6 (baseline, 5.50 ± 0.44 ppm and month 6, 6.00 ± 0.39 ppm; p=0.05). No such finding has been observed in the Se-group (baseline, 6.22 ±0.32 ppm and month 6, 6.03 ± 0.32 ppm; p=0.54).

3.5.4 Arsenic concentrations in erythrocyte

Arsenic levels in the blood are transient and primarily reflect recent exposure, As is rapidly redistributed into tissues, or excreted (192, 193). Erythrocyte As (Ery-As) levels were measured for subsamples (n=187) at baseline and at month 6. At baseline,Ery-As concentrations were at the same levels in the participants of control and Se-group. Between group comparison in Ery-As by two-way ANOVA or multivariable adjusted regression analysis showed no difference between the intervention groups at month 6. After 6 months of dietary intervention, Ery-As concentration remained unchanged within the Se-group, and there was no effect of sex. However, in the control group, Ery-As concentration was significantly increased in the females at month 6 (p=0.002) only compared to baseline (**Figure 3.2**).

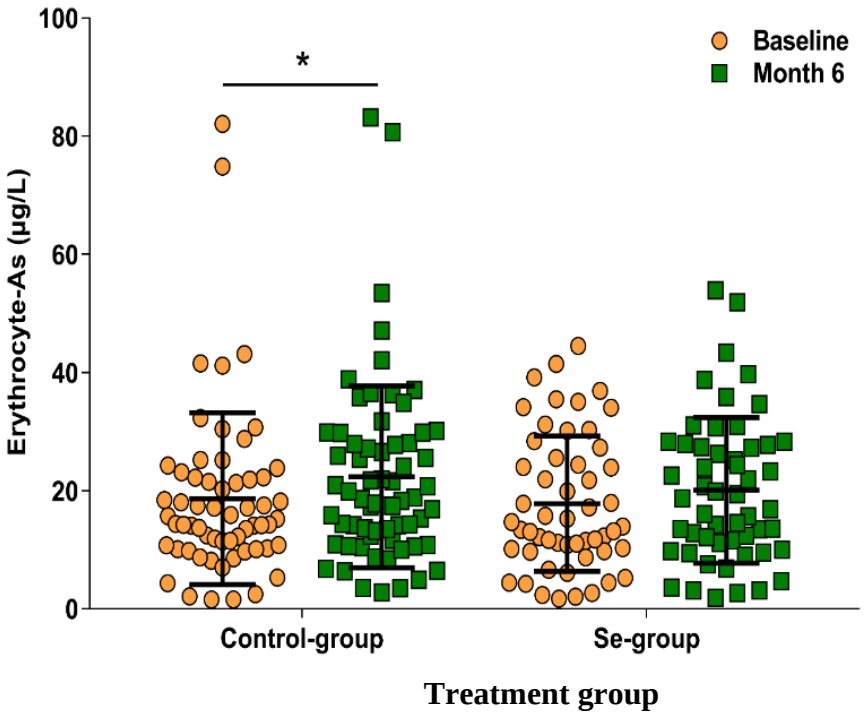

Figure 3.2 Erythrocyte As content within control and Se-group in the female participants only.

RDA dose of Se supplementation in lentil diet increased As excretion through urine however stool As excretion level remained unchanged after six months supplementation among As exposed participants.

Se-rich lentil supplementation for six months did not reduce the hair-As levels of the participants, though in females of Se-groups the dietary Se supplementation may regulate the hair-As content which was increased in the control group females (without having Se lentils) at month 6 compared to baseline.

In addition, dietary Se supplementation did not have any impact on Ery-As concentration in the As exposed population who were continuously consuming As

contaminated water. Similar to hair-As result, in the females of control group, the Ery-As levels were increased after six months which was not evident for Se-group's women.

3.5.5 Associations of arsenic concentration between different samples

The associations of As concentration between different samples have been assessed by multivariable regression analysis adjusted with covariates. Considering all participants from both groups, as expected, specific gravity adjusted U-As concentration (log) at baseline was strongly positively associated with water-As, stool-As, hair-As, and Ery-As level (**Table 3.7**).

Table 3.7 Associations of specific gravity adjusted urinary-As concentrations with other measures of As exposure, at baseline

Variables	**β[a] (95% CI)**	**p-value**
Water-As	56.1(16.5, 95.4)	**0.001**
Hair-As	3.86(2.82, 4.9)	**< 0.001**
Stool-As	273.2(176, 370.5)	**< 0.001**
Ery-As	21.33(17.2, 25.4)	**< 0.001**

[a]log of U-As concentrations are used for analysis. Multivariable adjusted regression model is used to estimate the p-value. Model is adjusted by age, sex, BMI at baseline, baseline water arsenic and house hold expenditure.

3.6 Selenium levels in urine and erythrocytes

Urine levels of Se (U-Se) were measured for a subset of participants (n=232) at all the three-time points. At baseline, there were no differences in the U-Se level between the two groups. While multivariable adjusted regression analysis indicated a significant increase in U-Se levels (log transformed) in the Se-group at month 3 and 6 compared to control group (Table3.8). However, to obtain within group difference between time comparison repeated

measure analysis was performed that showed a significant increase in U-Se levels in the Se-group at month 3 only (p=0.008) but not at month 6 (p=0.21) compared to baseline similar to U-As. In the control group, U-Se concentration remained unchanged over the 6 months of intervention (p=0.63 & 0.10 at months 3 and 6, respectively compared to baseline).

Table 3.8 Urinary specific gravity adjusted selenium concentrations at different time points

U-Se (µg/L)	Control group (n=116)	Se-group (n= 116)	β (95% CI)	p-value
At baseline	8.3± 1.1	8.8± 1.1	0.03(-0.05, 0.10)	0.52
At month 3	7.2± 1.07	10.2± 1.1	0.15 (0.06,0.24)	**0.001**
At month 6	7.2± 1.0	8.9± 1.0	0.09(0.02, 0.17)	**0.017**

Values shown are mean±SE; Multivariable adjusted regression model analysis is to calculate the p-value, CI (confidence interval). β -values are considered in Se-group compared to control. The model is adjusted by age, sex, BMI at baseline, baseline water arsenic, and household expenditure.

Like Ery-As, erythrocyte Se (Ery-Se) concentrations were analyzed for a subset of participants (n=200) at baseline and at month 6. Before supplementation, the Ery-Se levels were similar between the participants of control and Se-group. Between group comparison analysis indicated higher Ery-Se in the Se-group than the controls at month 6 (**Table 3.9**). The longitudinal GEE model analysis also demonstrated an overall increase in Ery-Se concentration in the Se-group after 6 months of dietary intervention compared to controls (β=30.04; 95% CI=7.58, 52.49; p=0.009). Repeated measure analysis also indicated increased levels of Ery-Se in the Se-group at month 6 compared to baseline (p <0.041) whereas in the control group, no such change was noted (**Figure 3.3**).

Table 3.9 Selenium levels in erythrocytes between the supplementation groups

Ery-Se (µg/L)	Control group (n=100)	Se-group (n= 100)	β (95% CI)	p-value
At baseline	119.1± 2.7	124.3± 2.7	6.12(-1.6, 13.9)	0.18
At month 6	125.7± 3.6	151.2± 3.7	23.68 (13.9, 33.5)	**<0.001**

Values shown are mean±SE; 2-way ANOVA is used to estimate the p-value. Multivariable adjusted regression model analysis is performed to calculate β-coefficient and 95% CI (confidence interval). β values are considered in Se-group compared to control. The model is adjusted by age, sex, BMI at baseline, baseline water arsenic, and household expenditure.

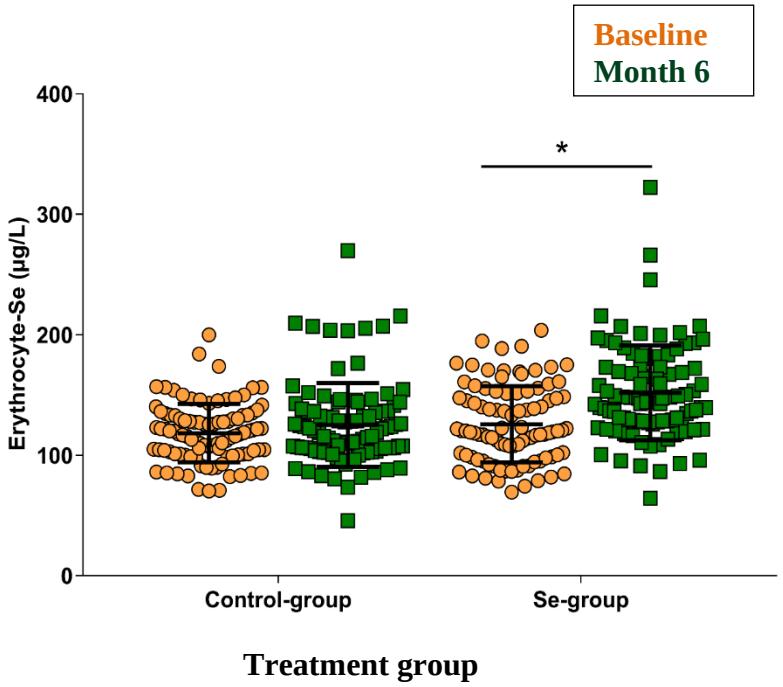

Figure 3.3 Within groups comparison of Ery-Se between time points by repeated measure analysis. Orange circles describe baseline, while green square represents month 6Ery-Se concentrations

Selenium rich lentil supplementation increased U-Se concentration since Se has the capacity to induce As excretion from the body after forming seleno-bis-arsenium ion complex while the supplementation increased the Ery-Se levels in the Se-group's participants after six month.

3.7 Associations between metals (arsenic and selenium) in different biological samples

The multivariable adjusted regression analysis has been performed considering all participants to determine the associations between As and Se in different samples at baseline. The concentrations of U-As and U-Se were significantly inversely associated at baseline (β=−4.13; 95% CI=−7.2, −1.05; p = 0.02) on the other hand, Ery-Se was significantly positively associated with U-Se (β=0.56; 95% CI=0.11, 1.0; p = 0.014) and Ery-As (β=0.40; 95% CI=0.05, 0.72; p=0.02).

At month 6, the associations between Ery-As and Ery-Se was observed individually within each group. Similar to the associations at baseline, Ery-Se level was significantly positively associated with Ery-As (p≤0.05) at the end of the supplementation separately in both groups, though Se rich lentil supplementation increased the Ery-Se levels only in Se-group's participants (Table 3.10).

Table 3.10 Associations of Ery-Se with U-Se, Ery-As, U-As metabolites at month 6

Variables	Control group		Se-group	
	β (95% CI)	p-value	β (95% CI)	p-value
U-Se*	-0.58(-1.58, 0.41)	0.25	-0.115(-1.34, 1.10)	0.85
U-As*	0.18(-0.73, 1.09)	0.69	0.30(-0.48, 1.08)	0.45
Ery-As	0.65(0.19, 1.120)	**0.007**	0.60(0.19,1.01)	**0.004**

*U-Se and U-As concentrations were adjusted with specific gravity of the urine. Multivariable adjusted regression model analysis is performed to calculate β-coefficient and 95% CI (confidence interval). Model is adjusted by age, sex, BMI at baseline, baseline water arsenic and house hold expenditure.

3.8 Changes in body mass index (BMI)

At baseline, BMI of the participants was measured. There was no difference in BMI between control and Se-groups' participants at baseline. Similarly, no differences were obtained in the BMI between the two groups at either month 3 or month 6 (**Table3.11**). When the participants were

categorized by age, for the adult participants (within 18-40 years of age)of Se-group had higher BMI at month 3 compared to that of control (β=0.26; 95% CI=−0.002, 0.52; p=0.05). For children (14-18 years old) and elderly people (>40 years), no such findings were noted. However, repeated measure analysis (comparison between time points i.e. from baseline to month 3 or from baseline to month 6) showed a significant increase in BMI within the Se-group from baseline to month 3(p<0.001), persisting up to month 6 (p=0.016)(**Figure 3.4**).When stratified by sex, BMI increased in both males and females at month 3(p=0.01, p=0.008, respectively), persisting only in males at month6 (p=0.04) in the Se-group. BMI remained unchanged within control group over the 6 months' intervention period (data not shown).

Table 3.11 Comparison in body mass index of the participants between the intervention groups

BMI(kg/m2)	Control group (n=201)	Se group (n=204)	β (95% CI)	p-Values
At baseline				
All	21.61±0.27	21.79±0.27	0.18 (-0.57, 0.93)	0.64
Male	20.59±0.39	20.80±0.34	0.21(-0.81, 1.23)	0.68
Female	22.30±0.37	22.48±0.40	0.18(-0.89, 1.25)	0.73
At Month 3				
All	21.61±0.28	22.08±0.28	0.47(-0.31, 1.25)	0.24
Male	20.55±0.38	21.07±0.34	0.52 (-0.51, 1.56)	0.32
Female	22.32±0.38	22.78±0.41	0.45(-0.66, 1.57)	0.42
At Month 6				
All	21.63±0.28	22.04±0.28	0.41(-0.38, 1.2)	0.31
Male	20.62±0.40	21.09±0.36	0.47(-0.60, 1.55)	0.39
Female	22.30±0.38	22.69±0.41	0.39(-0.71, 1.50)	0.48

Values are presented as mean±SE, Multivariable adjusted regression model analysis is performed to calculate β-coefficient, 95% CI (confidence interval), and p-values. β values are considered in Se-group compared to control. The model is adjusted by age, sex, BMI at baseline, baseline water arsenic, and household expenditure.

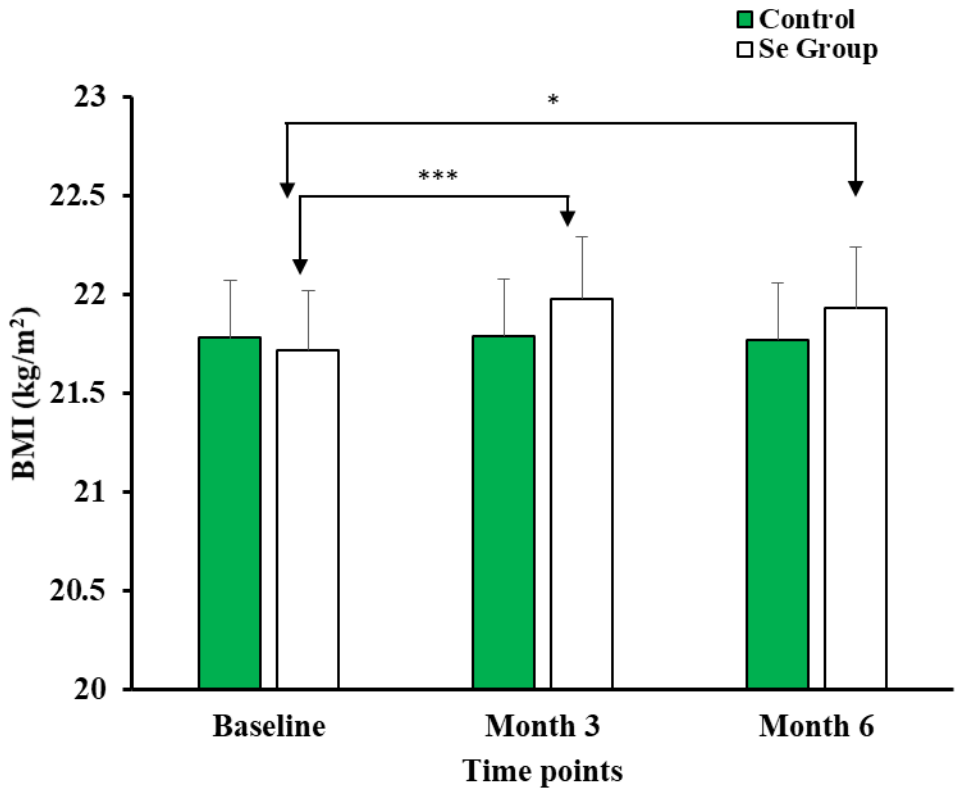

Figure 3.4 Changes in BMI in control and Se-group during the intervention period by repeated measure analysis adjusted with covariates. *** represents $p<0.001$ and * represents $p<0.05$.

Daily consumption of lentils containing RDA dose of Se for six months increased the BMI of the participants of Se- group over the study period whereas no change in BMI was observed among the control group participants consuming low Se lentil.

3.8.1 Effect of arsenic exposure and blood selenium levels on BMI

Multivariable adjusted regression analysis showed no effect of As exposure(via water and U-As) on BMI considering all participants from both groups at baseline as well as within either control or Se-group at any of the time points (**Table 3.12**). At baseline, no significant association was obtained between Ery-Se and BMI neither considering all participants nor in each individual group, while a positive association was obtained

between BMI and Ery-Se at month 6 in all participants prominent in the Se-group (p=0.001), but no such association was noted in the control group (**Table 3.12**).

Table 3.12 Associations of BMI with water-As, U-As and Ery-Se

Variables	All		Control-group		Se-group	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Water-As						
Baseline	-0.0001(-0.002, 0.002)	0.93	0.003(-.0012, 0.007)	0.156	-0.002(-0.005, 0.001)	0.197
Month 3	-0.0001(-0.003, 0.003)	0.96	0.004(-0.0001,0.009)	0.058	-0.003(-0.006, 0.0004)	0.085
Month 6	0.00002(-0.003, 0.003)	1.0	0.003(-0.001, 0.008)	0.118	-0.002(-0.006, 0.001)	0.213
U-As(log)						
Baseline	0.28 (-0.81, 1.36)	0.61	-0.14(-1.83, 1.55)	0.87	0.41(-1.00, 1.82)	0.57
Month 3	-0.10(-1.17, 0.96)	0.85	-0.80(-2.51, 0.90)	0.35	0.19(-1.13, 1.52)	0.78
Month 6	0.23(-0.88, 1.35)	0.68	-0.65(-2.41, 1.11)	0.46	0.63(-0.82, 2.08)	0.39
Ery-Se						
Baseline	0.36(-0.74, 1.45	0.52	0.12(-1.14, 1.4)	0.84	0.57(-1.42, 2.56)	0.57
Month 6	1.71(0.24, 3.19)	**0.02**	-0.28(-2.08, 1.52)	0.76	3.77(1.54, 5.98)	**0.001**

Multivariable adjusted regression model analysis is performed to calculate β-coefficient, 95% CI (confidence interval) and p-values. Model is adjusted by age, sex, BMI at baseline, baseline water arsenic and house hold expenditure.

Positive association between Ery-Se and BMI after six month of supplementation indicated the beneficial effect of Se rich diet to the As exposed population.

3.9 Morbidity outcomes

The morbidity information of the participants was collected biweekly using a structured questionnaire. The questionnaires included specific morbidity questions concerning the recent asthma incidences, skin problems, allergic skin diseases, respiratory illness (e.g. cold,

cough, or difficult breathing), urinary tract infections (i.e., pain, increased frequency, burning sensation, or difficulty during urination) with or without fever, and diarrhea/loose stool as well as the duration (days) of the morbidity symptoms. The multilevel, mixed effect, logistic regression analysis considering monthly morbidity outcomes revealed significantly lower risks of incidents of asthma and allergy in the Se-group compared to the control group. The odds of having asthma over the intervention period in the Se-group was reduced by 21% (p=0.05) and allergy by 18% (p=0.02) compared to controls (**Figure 3.5**).No other morbidity outcome was significantly reduced by the dietary intervention (**Table 3.13**) and neither was the effect of sex observed (data not shown).The analysis was performed considering potential confounders that affected the analysis by >5% (age, sex, BMI, and water-As concentration at baseline, household expenditures, and time).

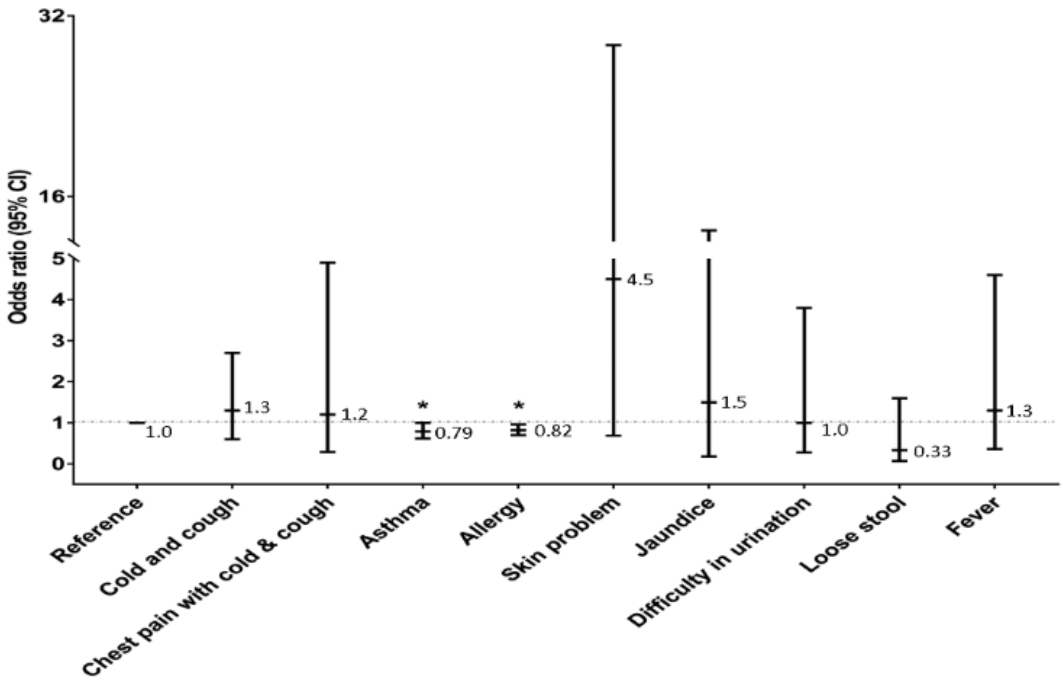

Figure. 3.5 Risk of incidences having morbidity outcomes in the Se-group compared to control group

Table 3.13 Morbidity outcomes in the Se-group compared to control

Variables	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted[a] Odds Ratio (95% CI)	p-value
Control group	**Ref**		**Ref**	
Cold and cough	1.0 (0.87, 1.2)	0.74	1.3 (0.60, 2.7)	0.53
Chest pain with cold and cough	0.92 (0.66, 1.3)	0.66	1.2 (0.29, 4.9)	0.81
Asthma	0.74 (0.53, 1.0)	0.067	0.79 (0.62, 1.0)	**0.05**
Allergy	0.81 (0.70, 0.96)	0.01	0.82 (0.70, 1.0)	**0.02**
Skin problems	1.3 (1.0, 1.6)	0.04	4.5 (0.69, 29.4)	0.11
Jaundice	1.1 (0.71, 1.8)	0.59	1.5 (0.18, 13.0)	0.70
Difficulty in urination	0.86 (0.67, 1.11)	0.26	1.0 (0.28, 3.8)	0.96
Loose stool	0.79 (0.53, 1.2)	0.24	0.33 (0.07, 1.6)	0.16
Fever	1.1 (0.80, 1.40)	0.68	1.3 (0.36, 4.6)	0.70

OR, Odds Ratio; CI, confidence interval. [a]Model is adjusted by age, sex, BMI at baseline, baseline water arsenic and house hold expenditure and time. p-values are estimated by multilevel mixed effect logistics regression model.

Se-bio fortified lentils consumption for six month reduced the incidences of some of the morbidity outcomes including asthma and allergy in participants of Se-group compared to control group.

3.10 Impact of dietary selenium supplementation on the levels of inflammatory biomarkers

3.10.1 Status of C-reactive protein in plasma

In the current study, we measured the CRP levels to assess the acute infection status of the participants. The concentrations of CRP in plasma at baseline were similar among the between the two groups participants. Multivariable adjusted regression analysis showed no difference in the levels of plasma CRP at months 3 and 6 between the 2 groups. There was no difference by sex in the CRP levels between the groups at either month 3 or 6 (**Table 3.14**). Repeated measure analysis also showed no differences in CRP

levels within either Se- or control group during the intervention period (from baseline to month 3 or 6) (data not shown).

For our As exposed study participants, plasma CRP levels were not associated with the U-As concentration at baseline, months 3 and 6. While stratified by groups, even then, no significant association was noted (data not shown).

Table 3.14 Comparison of plasma CRP levels between control and Se-group

CRP concentration (mg/L)	**Control group** (n=201)	**Se-group** (n= 204)	**β (95% CI)**	**p-value**
At baseline	2.08 ± 0.22	1.85 ± 0.22	-0.23 (-0.86, 0.39)	0.45
At month 3	2.36± 0.46	2.55 ± 0.42	0.19 (-1.09, 1.46)	0.77
At month 6	2.99± 0.72	2.84 ± 0.72	-0.15 (-2.18, 1.87)	0.88
For male	n=72	n=93		
At baseline	1.60 ± 0.24	1.49 ± 0.21	-0.11 (-0.74, 0.52)	0.73
At month 3	2.18 ± 1.08	2.87 ± 0.94	0.69 (-2. 17, 3.55)	0.63
At month 6	3.81 ± 1.83	3.64 ± 1.63	-0.17 (-5.04, 4.71)	0.94
For female	n=129	n=111		
At baseline	2.38 ± 0.33	2.11 ± 0.33	-0.27 (-1.23, 0.68)	0.57
At month 3	2.39 ± 0.33	2.35 ± 0.36	-0.41 (-1.01, 0.93)	0.93
At month 6	2.33 ± 0.27	2.46± 0.29	0.13 (-0.66, 0.92)	0.75

Data are presented as mean±SE, CRP, C-reactive protein; p-value has been determined by multivariable-adjusted regression model analysis. β values are considered in Se-group compared to the control group.The model is adjusted by age, sex, BMI at baseline, baseline water As, and household expenditure.

3.10.2 Fractionated nitric oxide levels in exhaled air

Fractionated nitric oxide (FE_{NO}) levels were measured in exhaled air as an indirect indicator of lung inflammation. According to ATS criteria, at baseline only 16 persons out of 383 participants had eosinophilic airway inflammation(FE_{NO}>35 ppb). There was no significant difference in FE_{NO} levels of the participants between the two groups at months

3 and 6 (**Table 3.15**). When we categorized the participants by sex, similar findings were obtained. Repeated measure analysis showed that the levels of FE_{NO} were decreased significantly in both the Se- and control group in a persistent manner from baseline to month 3 and also at month 6 ($p<0.05$) (**Figure 3.6**).

In this study, we did not find any effect of As exposure (considering water- and U-As concentration) on FE_{NO} levels at baseline considering both groups and either in any of the supplementation groups at any time points (data not shown).

Table 3.15 Between group comparison of fractionated nitric oxide levels in the exhaled air

FE_{NO} levels (ppb)	**Control group (n=186)**	**Se-group (n=197)**	**β (95% CI)**	**P- value**
At baseline	14.07 ± 0.75	14.15 ± 0.73	0.08 (-1.99, 2.16)	0.94
At month 3	12.99 ± 0.85	12.99 ± 0.83	0.69 (-1.66, 3.05)	0.56
At month 6	11.96 ± 1.46	10.99 ± 1.06	-0.98 (-4.62, 2.66)	0.60

Data are presented as mean±SE, p-values are estimated by multivariable-adjusted regression analysis. β values are considered in Se-group compared to the control group. Model is adjusted by age, sex, BMI at baseline, baseline water arsenic, and household expenditure.

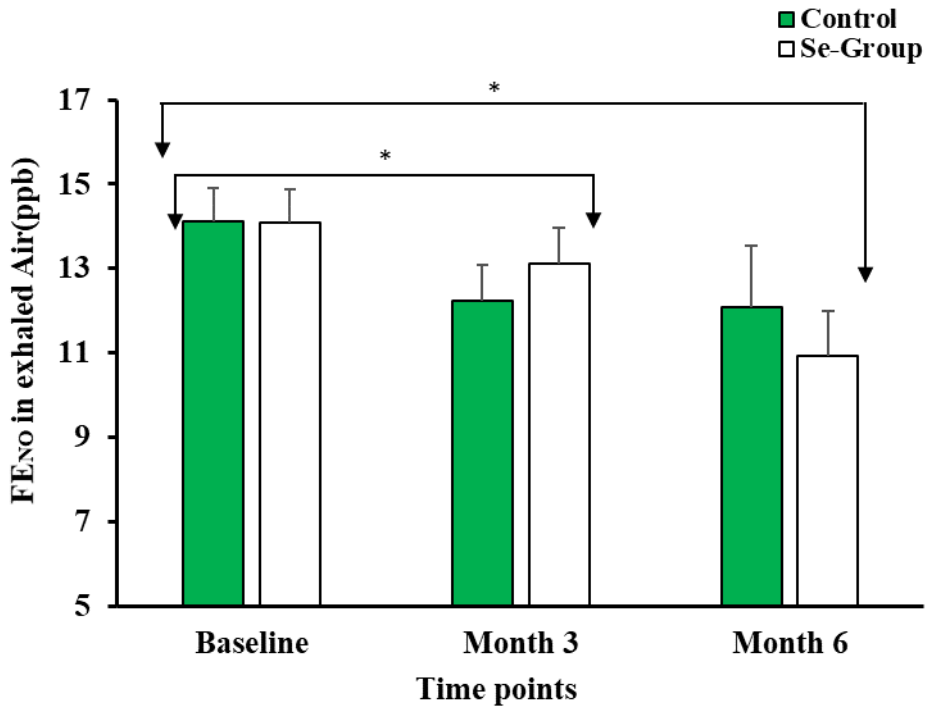

Figure 3.6 Changes in fractionated nitric oxide level in exhaled air within each group during the intervention period by repeated measure analysis adjusted with covariates

Dietary supplementation of Se did not have any impact to reduce the inflammatory markers, however with the high nutritional values lentil supplementation reduced the lung inflammatory marker over six-month period.

3.10.3 Associations of the concentrations of inflammatory biomarkers with incidences of asthma and cold-cough

As mentioned earlier, the incidence of having asthma and cold-cough related information had been collected from the participants fortnightly by using a structured questionnaire. Whereas, FE_{NO} levels in exhaled air and plasma-CRP concentrations were measured at baseline, month 3 and 6. The association between the levels of inflammatory biomarkers and the incidence of occurring asthma and cold-cough was evaluated by multivariable-adjusted regression analysis.

There was no significant association between plasma CRP levels and the incidence of asthma at any of the time points in the current As exposed study participants. However, plasma CRP level was higher among the participants having cold-cough compared to those not having cold-cough at baseline considering both groups. At month 3 for all participants as well as within each individual group, similar findings were noted (**Table 3.16**).

The levels of FE_{NO} were also higher in the participants with asthma compared to those without asthma at all-time points. An association between FE_{NO} and asthma was prominent only in the control group at months 3 and 6. Though at baseline there was no effect of cold-cough incidence on FE_{NO} level among all participants as well as separately within individual group. However, FE_{NO} levels were higher in the participants having cold cough compared to those not having that symptom only at month 3, when considering all participants as well as in control group only. Interestingly among the participants in Se-group such finding was noticed only at month 6 (**Table 3.16**). Male participants had higher FE_{NO} levels than that of females at baseline (β=3.65; 95%CI=1.49, 5.81; p=0.001), Month 3 (β=2.68; 95% CI=0.24, 5.13; p=0.032) and month 6 (β=2.32; 95% CI=-1.41, 6.05; p=0.22) considering all participants from both group.

Table 3.16 The levels of inflammatory markers in the participants with Asthma and cold-cough compared to those without any symptoms

Morbidity outcomes	**All** β (95% CI)	**p-value**	**Control** β (95% CI)	**p-value**	**Se-group** β (95% CI)	**p-value**
Plasma-CRP levels(mg/L)	**n=405**		**n=201**		**n=204**	
Asthma (Baseline)	-0.053(-1.06, 0.96)	0.92	-0.25(-1.52, 1.02)	0.70	0.09(-1.54, 1.72)	0.91
Asthma (month 3)	-1.49(-4.31, 1.34)	0.30	-0.86(-3.61, 1.88)	0.53	-2.10(-7.05, 2.84)	0.40
Asthma (month 6)	-1.54(-5.86, 2.78)	0.48	-1.08(-7.00, 4.83)	0.72	-3.52(-10.03, 2.99)	0.29
Cold and cough (Baseline)	0.78(0.16, 1.39)	**0.014**	0.23(-0.59, 1.06)	0.575	1.23(0.29, 2.17)	**0.01**
Cold and cough (month 3)	2.53(1.04, 4.01)	**0.001**	2.51(0.98, 4.03)	**0.001**	2.62(0.09, 5.13)	**0.04**
Cold and cough (month 6)	1.41(-8.88, 3.71)	0.22	2.88(-0.53, 6.28	0.10	-1.22(-4.37, 1.93)	0.45
FE_{NO} levels (ppb)	**n=383**		**n=186**		**n=197**	
Asthma (Baseline)	3.63(0.26, 7.00)	**0.03**	4.13(-0.98, 9.25)	0.11	3.53(-1.01, 8.08)	0.13
Asthma (month 3)	9.76(4.42, 15.12)	**<0.001**	15.46(7.92, 23.00)	**<0.001**	4.48(-3.14, 0.16)	0.25
Asthma (month 6)	9.96(3.32, 15.99)	**0.002**	13.75(1.71, 25.78)	**0.03**	5.02(-2.54, 12.59)	0.19
Cold and cough (Baseline)	0.45(-1.62, 2.52)	0.671	-0.02(-3.32, 3.28)	0.99	1.13(-1.58, 3.84)	0.41
Cold and cough (month 3)	4.35(1.62, 7.08)	**0.002**	5.81(1.45, 10.17)	**0.01**	2.87(-0.81, 6.55)	0.12
Cold and cough (month 6)	2.95(-0.76, 6.68)	0.12	-1.32((-1.03, 7.40)	0.76	4.41(0.39, 8.49)	**0.03**

Inflammatory markers levels in the participants without having asthma and cold-cough is reference. Data are presented as β (95% CI). p-values were calculated by multivariable adjusted regression model analysis. Model is adjusted by age, sex, BMI at baseline, baseline water arsenic and house hold expenditure.

We did not find any direct association between inflammatory markers level and the incidences of asthma and allergy in the participants. However, while the participants were categorized by with/without asthma and cold-cough, the participants with higher chances of occurrence of asthma and cold-cough had a higher levels of the inflammatory markers than the heathier ones.

3.11 Levels of oxidative stress marker and antioxidant in the blood

In the current study, plasma 8OHdG levels and total glutathione concentration in erythrocytes of the participants were measured at baseline and at month 6 to demonstrate the effect of Se-rich lentil supplementation on oxidative stress and antioxidant levels among As exposed population.

Multivariable adjusted regression model analysis showed that at baseline there was higher levels of plasma 8OHdG in the participants of Se-group compared to control group. Thus in the same analysis at month 6 to assess the effect of Se rich lentil supplementation, baseline 8OHdG level of the participants was used as one the covariates along with the other earlier mentioned covariates observing no significant change in the levels of 8OHdG at month 6 between the two groups of participants (**Table 3.17**). Interestingly, repeated measure analysis showed a significant decrease ($p=0.021$) in 8OHdG levels in the control group while in Se-group the plasma 8OHdG levels remained unchanged at month 6 compared to baseline ($p=0.34$).

. The levels of total glutathione (GSH) in blood of the participants (**Table 3.17**) were similar in the two groups at baseline and month 6. Whereas within group comparison by repeated measure analysis showed that, in both the Se- and control group, the total

GSH levels in erythrocytes were decreased significantly at month 6 compared to baseline ($p<0.001$).

Table 3.17 Comparison in 8OHdG and GSH levels between the Se- and control group

Variables	Control group	Se-group	β (95% CI)	p-value
8OHdG(nM)	n=107	n=90		
At baseline	73.68 ± 3.11	82.82 ± 3.40	8.93 (-0.22, 18.09)	0.06
At month 6	72.65 ± 2.07	77.51 ± 2.29	4.85 (-1.33, 11.04)	0.12
GSH(µM)	n=95	n=94		
At baseline	1434.35 ± 26.42	1456.23 ± 26.42	21.88 (-52.14, 95.90)	0.56
At month 6	1215.28 ± 26.32	1261.87 ± 26.46	46.59 (-27. 36, 120.54)	0.21

Data are given either as mean±SE, p-values were estimated by multivariable-adjusted regression analysis. β values are considered in Se-group compared to the control group. The model is adjusted by age, sex, BMI at baseline, baseline water arsenic, and household expenditure.

There was no effect of six months dietary Se supplementation (in RDA dose) for the population chronically exposed to higher levels of As (where the mean water As concentration was 466µg/L) in reducing the oxidative stress levels as well as to improve body's anti-oxidant levels.

In this study, we did not observe any impact of As exposure on plasma 8OHdG levels (for water As: β=-0.01; 95% CI=-0.04, 0.02; p=0.48& for U-As: β=4.39; 95% CI=-9.56, 18.34; p=0.54) at baseline. At month 6, a significant positive association was noted between U-As excretion and 8OHdG for all participants (β=19.24; 95% CI=3.72, 3.75; p=0.015) which was prominent in control group's participants (β=28.52; 95% CI=8.59, 48.45; p=0.005) but not in Se-group (β=7.50; 95% CI=-15.26, 30.26; p=0.514).

On the other hand, at baseline a strong inverse association was noted between U-As and GSH levels (β=-165.13; 95% CI=-302.35, -27.91; p=0.002) and among all participants (**Figure 3.7**). However, at month 6, a non-significant inverse association was obtained in all participants (β=-103.11; 95% CI=-210.72, 4.50; p=0.06) as well as separately in control group (β=-114.62; 95% CI=-276.70, 47.45; p=0.163), and Se-group (β=-104.74; 95% CI=-256.91, 47.42; p=0.17).

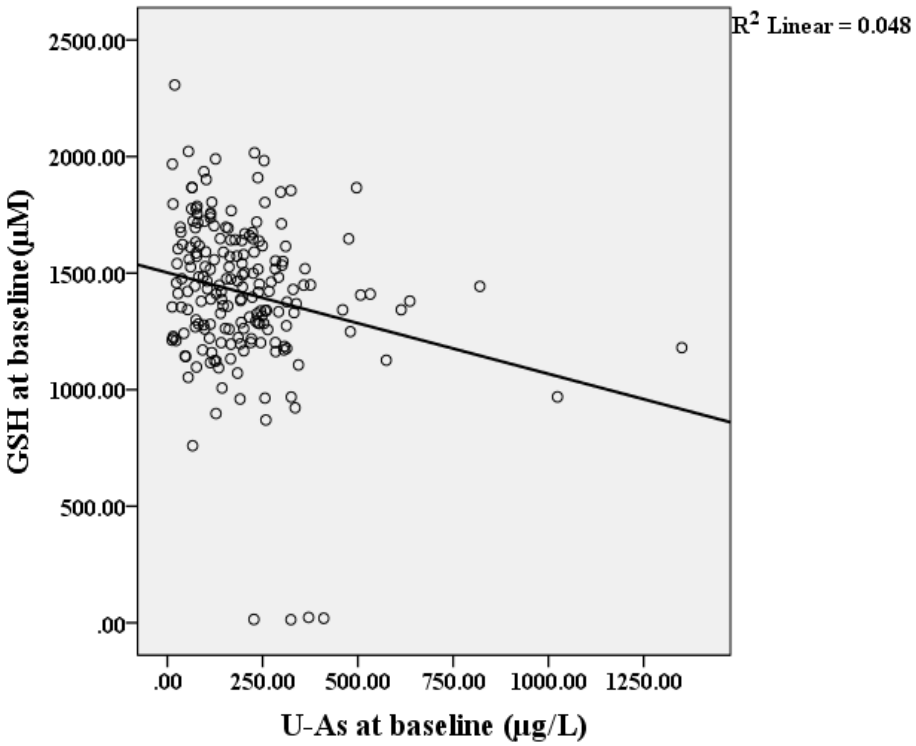

Figure 3.7: Association between adjusted Urinary As and erythrocyte glutathione levels at baseline

A significant positive association was found between GSH concentration and Ery-Se levels at baseline (β=3.04; 95% CI=1.39, 4.70; p=0.000) as well as at month 6 (β=1.00; 95% CI=0.03, 1.99; p=0.04) for all participants (**Figure 3.8 A and B respectively**).

Interestingly at month 6, no such association was obtained separately either in the control (β=1.40; 95% CI=-0.09, 2.89; p=0.07) or Se-group (β=0.23; 95% CI=-1.33, 1.78; p=0.77).

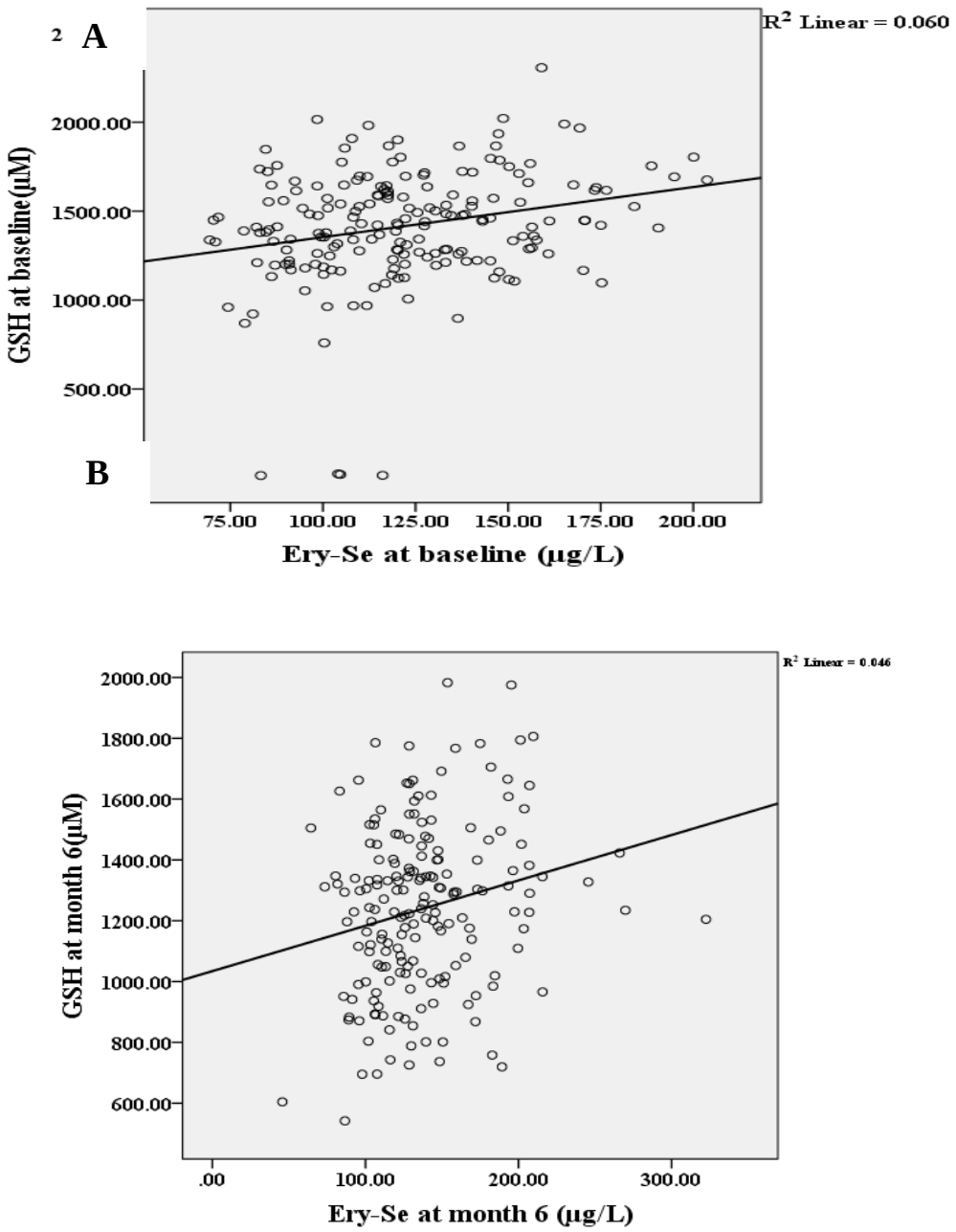

Figure 3.8 Association between erythrocyte Se and glutathione at baseline (A) and month 6 (B)

3.11.1 Blood pressure and lipid levels of the participants

Systolic and diastolic pressure levels and plasma lipids, including cholesterol (Chol), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglyceride (TG) concentration were measured before supplementation, at month 3 and 6.

Multivariable adjusted regression analysis indicated that Se-rich lentil supplementation did not have any impact on blood pressure (both systolic and diastolic pressure) and lipid levels among the current study participants. No significant change was observed in the systolic and diastolic blood pressure as well as plasma lipid levels at months 3 and 6 between the Se- and control group (**Table 3.18**).

Table 3.18 Comparison in blood pressure and lipids levels between the Se- and control group

Variables	Control group (n=201)	Se-group (n=204)	β (95% CI)	P- value
Systolic blood pressure (mmHg)				
At baseline	112.82±1.24	111.71±1.23	-1.11(-4.59, 2.37)	0.53
At month 3	106.74±1.13	105.84±1.12	-0.90 (-4.07, 2.27)	0.58
At month 6	109.02±1.00	108.63± 1.00	-0.39 (-3.21, 2.43)	0.78
Diastolic blood pressure (mmHg)				
At baseline	75.19 ± 0.77	75.14 ± 0.77	-0.056 (-2.21, 2.10)	0.96
At month 3	72.45±0.69	72.85±0.68	0.40 (-1.53, 2.33)	0.69
At month 6	73.66±0.66	73.08±0.65	-0.58 (-2.42, 1.26)	0.54
Chol (mg/dL)				
At baseline	146.36±2.45	144.28±2.43	-2.08(-8.92, 4.76)	0.55
At month 3	132.98±2.31	133.99±2.26	1.01(-5.41, 7.44)	0.76
At month 6	142.48±2.37	141.78±2.35	-0.71(-7.34, 5.93)	0.83
LDL (mg/dL)				
At baseline	97.91±2.04	96.46±2.02	-1.45(-7.15, 4.25)	0.61
At month 3	86.57±1.87	87.53±1.84	0.96(-4.26, 6.18)	0.72
At month 6	87.66±1.99	87.20±1.97	-0.46(-6.02, 5.11)	0.87
HDL (mg/dL)				
At baseline	31.76±0.58	31.36±0.57	-0.39 (-2.00, 1.22)	0.63
At month 3	30.28±0.60	29.55±0.59	-0.73(-2.42, 0.95)	0.39
At month 6	29.63±0.59	29.94±0.59	0.31 (-1.35, 1.97)	0.72
TG (mg/dL)				
At baseline	115.20±4.98	116.38±4.94	1.17 (-12.75, 15.10)	0.87
At month 3	111.09±5.46	116.90±5.35	5.81 (-9.39, 21.01)	0.45
At month 6	123.85±5.64	121.21±5.59	-2.64(-18.41, 13.21)	0.74

Values are given as mean±SE, p-values were estimated by multivariable-adjusted regression analysis. Model is adjusted by age, sex, BMI at baseline, baseline water arsenic, and household expenditure.

However, repeated measure analysis showed that within both the Se- and the control group, the levels of blood pressure and lipids (except TG) decreased significantly at months 3 and 6 ($p<0.05$) compared to baseline (**Table 3.19**).

Table 3.19 Within group changes in blood pressure and lipids

Outcomes	**Baseline**	**Month-3**	**[a]P value**	**Month 6**	**[b]P value**
Systolic BP(All)	112.46±0.88	106.18±0.79	<0.001	108.82±0.71	<0.001
Control-group	113.13±1.37	106.86±1.00	<0.001	109.09±0.99	0.001
Se-group	111.79±1.12	105.50±1.22	<0.001	108.56±1.02	0.01
Diastolic BP(All)	75.26±0.54	72.56±0.48	<0.001	73.39±0.46	<0.001
Control-group	75.34±0.79	72.41±0.65	<0.001	73.74±0.66	0.05
Se-group	75.19±0.75	72.70±0.71	<0.001	73.04±0.65	0.001
Chol(All)	146.14±1.54	133.68±1.46	<0.001	142.36±1.53	0.01
Control-group	151.89±2.58	139.75± 2.53	<0.001	147.98±2.48	0.07
Se-group	140.4±2.02	127.61±1.83	<0.001	136.73±2.04	0.07
LDL(All)	97.75±1.31	87.18±1.12	<0.001	87.66±1.31	<0.001
Control-group	102.93±1.97	92.13±1.87	<0.001	92.87±1.80	<0.001
Se-group	92.58±1.67	82.24±1.86	<0.001	82.44±1.86	<0.001
HDL(All)	31.44±0.40	29.82±0.39	<0.001	29.83±0.40	<0.001
Control-group	31.97±0.58	30.80±0.58	0.001	30.28±0.60	0.002
Se-group	30.90±0.54	29.56±0.54	0.007	29.39±0.53	0.002
TG(All)	117.55±3.35	114.42±3.51	0.27	122.75±3.77	0.11
Control-group	121.98±5.35	118.41±5.38	0.42	125.94±6.01	0.45
Se-group	113.13±4.10	110.43±4.55	0.45	119.56±4.49	0.10

Data presented as mean±SE, p values were calculated by repeated measure analysis, [a] changes between baseline and month 3, [b] changes between baseline and month 6, model is adjusted by age, sex, BMI at baseline, baseline water arsenic and household expenditure.

In our study, we did not obtain any direct effect of As exposure on blood pressure and lipids levels at baseline and months 3 and 6. Multivariable adjusted regression analysis showed that there was no significant association of water and U-As with either blood pressure and lipids (data not shown) levels.

There was no positive impact of Se-lich lentil supplementation was noticed in the current population instead lentil supplementation for six months regulated the levels of biomarkers of cardiovascular diseases.

In summary, the supplementation of high Se containing lentils into the everyday diet providing RDA level of Se for six months to the As exposed rural Bangladeshi population was associated with higher excretion of As through urine mostly during the first three month of dietary supplementation depending on the participant's daily lentil intake compliance. It is also linked with increased levels of Se in erythrocyte, improved BMI and thereby reduced the incidences of chronic As exposure-related morbidity outcomes in the exposed population in Bangladesh.

Chapter 4

DISCUSSION

This thesis described the significant findings of a randomized, double-blind placebo control study showing a new approach to reduce chronic arsenic (As) exposure on adverse health effects in an As exposed rural Bangladeshi population by supplementing Se enriched lentil. For Bangladeshi people, lentil soup or dahl is one of the most common foods usually consumed daily. Lentils are a large source of plant-based protein, fiber, and it is also an excellent source of B vitamins, iron, magnesium, potassium, and zinc. People in Bangladesh and in the subcontinent take lentils in various preparations (i.e., soup, mashed lentils, a fried patty with mashed lentils, etc.) to fulfill the demands of daily proteins. Selenium is an antioxidant with considerable importance for human and animal health. This study demonstrated that Se-rich lentil supplementation to the highly As exposed rural Bangladeshi population for 6 months is associated with increased urinary As excretion, improved erythrocyte Se status. The study also depicted beneficial effects in selected health indicators (measured by BMI & morbidity outcomes). Furthermore, this study showed positive effects of lentil supplementation to control blood pressure and lipids, regardless of the Se levels in lentils.

In this study, a Se bio-fortified food source, i.e., lentils, was used for the very first time as an effort to reduce the adverse health effects of chronic As poisoning. Lentils are an outstanding source of non-animal protein and micronutrients such as Zn and Fe(194); thus, one of our anticipated outcomes was to observe improved BMI as well as to improve overall health status in all participants of the study. Animal studies showed that Se intervention along with As could form a comparatively non-toxic complex in the liver, readily excreted from the body, and thereby reduce As toxicity (114). To our knowledge, none of the recent human trials the evaluated the role of Se pills in the treatment of

arsenicosis have published their findings yet (195, 196). However, several trials with Se supplementation, alone or in combination with other micronutrients reported mild beneficial effects, however those studies were different from ours because challenges with compliance, study design, or small sample size (105, 108, 122, 197). In the current study, we expected to a reduction of As exposure-related adverse health effects evaluated by increased levels of As excretion through urine and stool as well as a remarkable increase in blood Se levels in the Se-arm.

Selenium is a necessary nutrient with a low 'therapeutic index', i.e., a narrow window between the useful and toxic doses (2, 198). For the current study, possibility of Se toxicity by over-supplementation (which could occur with pills or mineral mixtures) would be eluded as here Se is consumed via naturally cultivated food, lentils rather than manufactured sources (4). The naturally bio-fortified lentils in this trial contained Se levels of 0.85 mg/kg and delivered the RDA (i.e., 55µg Se/day) of Se with essentially has no risk of toxicity (174). Except for Se, the two varieties of lentils used in this study had similar levels of other macro- and micronutrient contents as well as the anti-nutrient, phytic acid levels (**Table 3.2**). The remaining lentils were reanalyzed at the completion of the trial after one and a half year from grown and slightly different levels of essential micronutrients and folate were marked (**Table 3.3**), approving the constancy of nutrients levels in lentils. The daily lentil intake compliance of the was similar for the two groups; >90% of participants ate the required amount of lentils in the first 3 months, with the compliance rate decreasing to ~80% in the subsequent 3 months.

The anticipated outcomes of this current study were that RDA level of Se in diet would result in higher blood Se and would form complex with free As and lead to

exposure reduction by increased As excretion through urinary (109, 198) and biliary routes (2, 109, 114-116), as well as decreased deposition of As in hair (105). Exposure reduction would be associated with health outcomes (measured by improved health benefits resulting increased BMI and reduced morbidity (such as asthma, allergy etc.), reduced inflammation (66) (assessed by plasma CRP, fractionated nitric oxide level in exhaled air to determine the particularly lung inflammation), and oxidative stress (199) (measured by plasma 8OHdG level). Additionally, lentil supplementation regardless Se concentration would expect to affect blood antioxidant levels (by erythrocytes total glutathione) (200) and regulate blood pressure and lipids (201, 202).

4.1 Water arsenic levels in the study participants

From a previous district-wise survey in Bangladesh, it has been reported that in Chandpur district, As contamination has taken serious turns. Our study area, Shaharasti, is one of the Upazila in Chandpur district. According to that survey, almost all the tube-well (98%) in Shahrasti Upazila were As contaminated (203). Initially, we evaluated water-As concentrations from tube wells of different households at the time of screening of the participants by using a semi-quantitative field kit to identify those consuming >100µg/L of As containing drinking water, while during the trial household tube well water was collected again to measure total As concentrations more accurately by atomic absorption spectrophotometry (AAS) (**Table 3.1**). The values of water-As measured by the AAS method were within the ranges obtained from the semi-quantitative analysis for all the tube wells. Despite taking several motivational and mitigation approaches (installation of deep tube wells, rain water harvesting, etc.) conducted by government or non-

governmental organizations, we found that at Shahrasti, more than 80% of the screened tube wells water (from 161 HHs) contained relatively very high levels of As (> 100 µg/L). In this study, we found that only 15 (7.4%) out of 161 tested tube wells' water had As content below the national guideline (<50 µg/L) while 131 tube wells (80.3%) had very high As content (>100 µg/L). To obtain increased possibility of detecting clinical changes between the two treatment groups, we selected families with tube-wells having As levels ≥100 µg/L. Thus, our study participants were exposed to very high levels of As through their drinking water.

4.2 Impacts of Selenium-biofortified lentil supplementation on excretion and deposition of arsenic

In this study, urinary-As (U-As) concentrations were assessed to describe the exposure as well as the excretion levels of As from the body. Urinary As concentration was used as a biomarker of As exposure. Arsenic is absorbed by the gastrointestinal tract and excreted through urine within a few days. If individuals are exposed daily through water and food, the excretion of As and its metabolites through urine likely reach a steady-state level. Therefore, U-As concentration reflects ongoing exposure to inorganic As from all sources, i.e., drinking water and food (64).

In this study, we found increased U-As excretion for the Se-group's participants, consuming RDA level of Se (55 µg/day) from naturally bio-fortified lentils at only months 3 but not at month 6 (**Table 3.4**) which may be resulted for the reduced lentil intake compliance of the participants during the last three month compared to first three months of supplementation. For the first three months of supplementation, >90% participants from

both group took the required amount (65gm/day) of lentil, however, the compliance of lentil intake dropped to 80% in the last three months may be due to the recommended amount was relatively higher than usual daily consumption by Bangladeshi people. In this trail, we used Se-rich lentil (for Se-group participants) which grown naturally in the soil of Lucky Lake, Saskatchewan, Canada where the Se-content was relatively very high. The average content of Se in Saskatchewan lentils is between 425–672 μg/kg, moreover in some areas as high as 1884 μg/kg(181). Initially we anticipated that in the naturally grown lentil, there would be the Se content > 1.5 ppm thus the study participants would obtain the RDA level of Se (55μg/day) from ~35 gm of lentils. However, after production it was found that the Se content of the lentils was 0.85 ppm (half of the anticipated content of Se in lentils). So we had to increase the amount of lentil for daily consumption to provide the RDA level of Se to Se-group participants. Thus from the dietary intervention, the participants from the Se-group consumed Se through lentils in the RDA dose which resulted, overall higher excretion of As through urine (all the three time point by longitudinal generalized estimating equation model analysis) in the compared to control that was prominent in females) (**Table 3.5).**

In our study we did not find any indication allowing us to convey the message that, bile as a crucial route of excretion for As in humans, as there was no notable change in stool-As concentrations observed over time (**Table 3.6**). In line with our finding, Vahter et al. also showed that urine is the main route of As excretion, not stool (48). Though a number of animal studies using rabbits and rats, have identified a complex of As and Se, seleno-bis-arsenium ion [(GS)2AsSe−] in the bile instead of in urine (48, 114, 115), detection of this or a similar complex has not been reported in humans. To our knowledge,

till now, no study in humans examining stool excretion of As, with or without Se supplementation, has been published. Thus this area of research needs further investigation.

In this study, hair-As concentration has been used as a non-invasive surrogate to reflect As absorption from the diet. However, we did not obtain any reduction in hair-As level in the Se-group after 6 months of intervention, most likely because of continued As exposure. Still, there was a clear trend towards increasing hair-As over the course of the trial only in the females of the control group. For this study, some protective factors may prevent further accumulation of As in the women's hair in the Se-group. The females of the Se-group also had the same As exposure as the controls but the consumption of RDA level of Se through lentil may prevent the further accumulation of As in hair while contributing to the health benefits to them. On the other hand, blood-As content has also been used as one of the reliable indicators of current As exposure (192, 204, 205). We found no changes in the Ery-As content between the participants of the two dietary groups.

4.3 Selenium concentrations in urine and erythrocytes

Blood Se concentration (whole, plasma, or serum) is used as the most informative biomarker of Se status at both the individual and population level (132, 206, 207). Since Se is incorporated during erythrocyte synthesis, Ery-Se reflects long-term Se consumption while U-Se indicates recent dietary intake (208). In the current study, an increased U-Se (**Table 3.8**) levels at both month 3 and 6, as well as Ery-Se (**Table 3.9**) concentrations after supplementation at month-6, were found in those participants who were particularly

on the Se-rich diet (**Figure 3.3**) even though they continued to be exposed to high As levels through drinking water.

4.4 Associations between selenium and arsenic in various biological samples

We found a strong positive correlation between Se and As in erythrocytes for both the groups separately (**Table 3.10**), reflecting that Se was appeared to move with As into the red blood cells, which further supported by Gailer et. al. showing binding of Se with As in erythrocytes (209). To observe the kinetics of intravenously (IV) administered As and Se in whole blood, erythrocytes, and plasma, a rabbit model was used (192). Within 5 min of IV injection of selenite and arsenite, 99% of the As was bound in the erythrocytes, largely as seleno-bis-(S-glutathionyl) arsinium ion [(GS)2AsSe]-, with a negligible amount in plasma. After forty minutes, the whole blood As concentration became less than half, and by 24 h, there was no detectable blood As representing the rapid and profound interaction between As and Se, as well as the mobility of these compounds and suggesting that erythrocytes are essential for this route of excretion of As. Detailed mechanistic studies would reveal these proposed relationships, particularly with chronic As exposure.

In the body, homeostasis of Se is centrally maintained and regulated by the liver and predominantly excreted via urine and feces (210, 211). During the excessive requirement of the body for Se, the liver reduces Se excretion, using it to synthesize vital selenoproteins. It is still unclear by which regulatory mechanism the ingested Se is allocated to the retention or excretion processes in the body. Antagonizing the toxic effects of As by Se in humans may occur through various pathways that include mediation through erythrocytes (211).

4.5 Dietary selenium supplementation improves BMI and ensures health benefits to As exposed population

Various studies confirmed the associations between the harmful effects of As exposure and malnutrition (63, 64, 212, 213), which is an additional, prominent health concern in Bangladesh, especially in children (214). As lentils are an excellent source of protein and micronutrients such as zinc and iron (180), we anticipated obtaining improved BMI in all the participants after dietary intervention compared to baseline, regardless of dietary Se content. However, the expected improvement has occurred only in the Se-group may be due to the higher excretion of As through urine **(Figure 3.4).** Daily Se-rich lentil consumption may restrict to deposit of the currently consumed As in the body and thereby reducing As related health problems during the study period (i.e., lowering the incidence of Asthma and allergy during the study period) and finally improved BMI of the participants of Se-group. Whereas for the control group's participants, as the U-As excretion remained almost similar all through the intervention period additionally due to continuous consumption of high As containing water, the improvement of BMI may not be evident.

The pathogenesis of asthma is mainly induced by the formation of oxidative stress. Selenium plays its leading role as an antioxidant. Thus, in terms of quantifiable health benefits, it is logical for this study to consider that the higher blood-Se content in the high-Se group participants plays a role in counteracting As-induced oxidative stress, reducing the risk of allergy and asthma problems (**Table 3.12 & Figure 3.5**). Selenium supplementation was found to be beneficial by reducing allergic airway inflammation in rodents (215). In another study in rats, Sah et al. showed the immunological influence of Se-treatment against As exposure evaluated by reversed As-induced antibody-mediated

immunosuppression, as well as hepatic lipid peroxidative damage (116). The protective benefits of Se in As exposed animals were found in the later studies, where mostly higher Se doses were given with four times the Se RDA. This level of Se falls within the safe range for humans also (216, 217). From epidemiological studies, it has been reported that low levels of Se were associated with increased incidence, prevalence, or severity of asthma, although interventional studies present conflicting results regarding Se status and asthma (66, 218, 219).

4.6 Effects of dietary Se supplementation on inflammatory biomarkers

Both experimental and epidemiological studies showed associations between As exposure and higher levels of various inflammatory markers including acute phase protein, pro-inflammatory cytokines (139, 141-144). CRP has been used as one of the most commonly used inflammatory biomarkers (151). There are numerous causes of elevated CRP. These include acute and chronic conditions, and these can be infectious or non-infectious in etiology. However, markedly elevated CRP levels are most often associated with infection(152). Among Bangladeshi adults, plasma CRP levels were found to be positively associated with water As (mean±SD 138±124 μg/L, p=0.01), urinary-As (232±205μg/L, p=0.12) and blood As (13±10 μg/L, p=0.07) concentration (143). In contrast, for our study participants who were also consuming very high levels of As through water (mean±SD, 442.3±125.3 and 466.2±150.7 μg/L for control and Se-group respectively), we did not obtain any significant association between As exposure (water, urine) and plasma CRP levels.

While the association between blood Se and CRP levels was reviewed, a mixed result has been found (220, 221). A case-control study among 64 comatose patients

showed no association between plasma Se and CRP concentration (220). In a prospective observational study among patients with respiratory diseases, a weak inverse correlation was noted between Se and CRP (221). In line with that, in the current study, plasma CRP concentration was inversely associated (β=-5.88; 95% CI=-9.85, -1.9; p=0.004) with erythrocyte-Se (Ery-Se) content at baseline all participants. However, after supplementation at month 6, the significant inverse association was noted only in the control group (β=-5.88; 95% CI=-9.85, -1.9; p=0.004), in Se-group, such finding was not observed that might be due to the beneficial role of Se which was increased (as Ery-Se) only among the participants of Se-group only.

On the other hand, the fraction of exhaled NO (FE_{NO}) is a promising biomarker for diagnosis, follow-up, and a guide to therapy in adults and children with asthma. The breath test has recently become available in many well-equipped hospitals in developed countries, although its exact role remains unclear.

In a mother-child longitudinal cohort study among chronically As exposed Bangladeshi pre-adolescent children, no significant association was observed between U-As and FE_{NO} levels(218).Similar to that study, in the current trial we also found no significant association between U-As and FE_{NO} levels neither in all participants nor within any individual group at any of the time points. According to ATS criteria, in our study, at baseline, only 16 participants had higher FE_{NO} levels as recommended for the normal subjects (having no eosinophilic airway inflammation), i.e., <35 ppb (222). At the end of the current supplementation trial, the frequency of normal FE_{NO} levels (<35 ppb) among all the study participants as well as for each intervention group was similar to the baseline. In an adult community-based study, Travers et al. showed that FE_{NO} levels for males were

higher than females (223). Like that study, we also found significantly higher FE_{NO} levels in males than that of females at baseline (β=3.65; 95% CI=1.49, 5.81; p=0.001) and at month 3 (β=2.68; 95% CI=0.24, 5.13; p=0.032) considering all participants. Travers et.al. further showed that FE_{NO} levels of the participants with asthma were even higher (p<0.001) than in those with no asthma, thus higher levels of FE_{NO} for the asthemic subjects (223). In line with that study, we also found higher FE_{NO} levels for the participants with asthma and cold-cough than those without these symptoms (**Table 3.16**).

In a randomized, double-blind, placebo-controlled clinical trial among patients with diabetic nephropathy showed that, 200 µg/d Se supplements as Se yeast for 12 weeks had favorable effects on plasma NO levels (p=0.04) (224). In the current study, we did not observe any effect of Se-supplementation on the nitric oxide levels exhaled air. To the best of our knowledge, till date no study in human was found to evaluate the effect of Se supplementation on the lung inflammatory markers particularly FE_{NO} levels.

Though RDA level of Se supplementation did not have any direct impact on the CRP (**Table 3.14**) and FE_{NO} levels (**Table 3.15**) between the two intervention groups, within-group comparison showed that lentil supplementation significantly reduced FE_{NO} levels at both months 3 and 6 compared to baseline for all participants as well as for both individual group (control and Se group) (**Figure 3.6**). It is well established that the consumption of lentils is massively connected in reducing the incidence of diseases such as diabetes, obesity, cancers, and cardiovascular diseases due to its bioactive compounds(225). Lentil is a good source of different macro- and micronutrients, including folate, zinc, iron, etc. (226). Zinc deficiency is found to be associated with elevating inflammatory response as well as damaging host tissue. Zinc is also involved in regulating

oxidative stress and inflammatory responses (227). One the other hand, the other micronutrient folate is used to prevent endothelium dysfunction by maintaining the levels of nitric oxide (NO) (228). Thus, Folate, Zn, and other nutrients, regardless Se content in both lentil diets, may have contributed to regulate excessive inflammatory responses in various chronic diseases for the As exposed rural Bangladeshi participants.

4.7 Impact of Se supplementation on oxidative stress biomarker and antioxidant levels in the blood

Arsenic exposure is known to induce reactive oxygen species' production leading to oxidative stress and DNA damage (133), assessed by determining 8-hydroxy-2′-deoxyguanosine (8OHdG)concentration, a known biomarker of oxidative DNA damage. Earlier ,a number of studies with animal models and cell lines showed the protective ability of Se (as selenite or Se from lentils) against arsenite-induced hepatic oxidative damage (109, 229-231). Xu et al. showed that administration of Se in the form of selenite (1.61–2.49 mg kg−1 body weight day−1) for 20-week to As exposed rat (1.24–1.90 mg kg−1 body weight day−1) through drinking water significantly reduced lipid peroxidation and restored the activity of glutathione peroxidase in the liver(231). Studies with rat model showed the protective effect of Se against As toxicity, suggesting that As exposure enhanced oxidative stress by disturbing the tissue antioxidant defense system, Se co-administration protected liver tissues against As intoxication via its antioxidant properties (109, 229).

Earlier a number of animal studies showed the beneficial effects of Se-supplementation in maintaining a normal pool of glutathione in the liver and increased

glutathione peroxidase concentration as well as in elevating the blood GSH level in As exposure (116, 232). In a human trail, among the patient with congestive heart failure, Se supplementation showed a significant elevation in plasma total glutathione levels compared to placebo (138). In the current study, six-month supplementation of Se-enriched lentils showed no significant difference in plasma oxidative stress marker (8OHdG) and blood antioxidant (total glutathione or Ery-GSH) levels between the two intervention groups' participants (**Table 3.17**).

Interestingly, within each group comparison between time points by repeated measure analysis showed a significant decrease in 8-OH-dG levels at month 6 compared to baseline only in the control group whereas the plasma 8OGdG levels were also decrease in the Se-group which was not significant. At baseline, the 8OHdG level was much higher in the participants of Se-group compared to control. As well as during the study period the participants from Se-group were taking tube well water with higher levels of As ($p=0.08$) compared control. Thus though the participants of Se- group were consuming Se-rich lentil diet, the Se-lentil provide only the RDA dose which might not adequate to reduce the 8OHdG in a significant level during the six months of intervention period. While within group comparison analysis also showed a significant decrease in total Ery-GSH levels over the 6 months among all participants as well as separately within each of the two groups. Both Se and As use glutathione for their metabolism which might cause the reduction of total glutathione levels after sis month of supplementation. We could not find any published research in humans that examined the effect of Se-supplementation on blood total GSH levels (in erythrocyte) and 8OHdG concentration (in plasma) in As exposed population, that area warrants further investigation.

Earlier it has been evidenced that in chronically As exposed Taiwanese population blood As level was positively correlated with reactive oxidants species (r=0.41, p=0.001) and negatively correlated (r=-0.30, p=0.014) with plasma antioxidant capacity (233). In line with that study, we found a significant positive association between U-As and 8-OH-dG at month 6 but not at baseline for all participants as well as for the participants of the control group. An inverse association was noted between U-As and Ery-GSH concentration considering all the study participants at baseline (p=0.002) but not at the end of the supplementation (p=0.06). As Se supplementation was associated with increased excretion of As during the 6 month of intervention period, thus at month 6, we might not obtain the expected trend of association.

4.8 Impacts of Se supplementation on blood pressure and plasma lipids

There were mostly conflicting results between the observational studies and randomized controlled trials (RCTs). Though a number of retrospective case–control studies showed that the blood Se concentrations of cardiovascular diseases (CVD) patients were lower than those of the healthy population, indicating an inverse correlation(175, 176).On the other hand, a few randomized trials have evaluated the effects of Se on cardiovascular outcomes(234-237)showing no apparent benefits from Se for CVD. In addition to heterogeneity in intervention periods and Se formula and dosage, these individual trials are limited by statistical power for addressing specific thresholds of circulating Se concentrations for optimal cardiovascular health. Previously, neither meta-analysis of six RCTs(175) for Se-containing supplements nor a meta-analysis of 12 RCTs for Se supplements alone(238)showed a significant protective effect on cardiovascular end

points. In a moderately As exposed mouse model, Se-methionine (Se-Met) supplementation maintained a normal pool of glutathione in blood, increased glutathione peroxidase (antioxidant) concentration in the liver and thereby reduced the chance of developing atherosclerotic lesion (232). For our study participants, we did not observe any significant change in the markers of cardiovascular diseases, including blood pressure and lipids (**Table 3.18**) after RDA dose of Se supplementation through lentils for 6 months' period. However, daily consumption of higher amount of lentils regardless of Se content improved lipid profile in both control and Se-group by decreasing blood pressure and plasma lipids (except for TG) (**Table 3.18**), highlighting the potential health benefits regular consumption of lentils.

Though epidemiological and experimental studies support the likelihood that As plays a significant role in the progression of hypertension and other cardio metabolic diseases (21, 162-164), a number of human trials showed different findings. A cross-sectional study comparing serum lipids in low levels of As exposed workers (where U-As levels were13.4±6.1 and 4.4±6.1µg/g Creat, for As exposed and control workers, respectively) showed no significant differences in chol, TG, HDL, and LDL concentrations between As exposed and control group (239). Another study among 225 northern Argentinean women showed that As exposure (median U-As concentration was 200 µg/L, ranged 22–545 µg/L)was not associated with elevated levels of early risk markers for cardiovascular disease(240). Similar to this study, we did not obtain any impact of As exposure (both water and U-As) on plasma lipid level though all the participants were consuming high levels of As (>100µg/L) through drinking water. We

also did not find any association between As exposure (for both water- and U-As concentrations) and blood pressure levels (both SBP and DBP) of the study participants.

Strengths of the current study included long-term, continued exposure of the population to As during the trial period, a low drop-out rate, high compliance, active monitoring of consumption of the supplied diet (lentils).

Limitations of the current study are that; (1) A subsample of the study subjects has been participated in stool collection, although stool As analysis revealed no differences between the groups or over time. Contrasting with animals, bile may not be an essential route of As excretion in humans. Alternatively, this may reflect the lack of research into this question; (2) many men worked away from the villages during the day. Similarly, children were in school, possibly affecting our As exposure assumptions; (3) We did not collect data on other dietary intake of Se-rich foods, which might influence the Se status. There might be slight day-to-day variation in dietary intake of Se between individuals. However, substantial differences in the intake of Se-containing food were not expected between the two groups over a period of 6 months; (4) for stool-As, Ery-Se, Ery-As, plasma 8OHdG and Ery-GSH analysis sub-samples were assessed. For stool As analysis, all the available samples were analyzed for As measurement. For Ery-Se, Ery-As, plasma 8OHdG and Ery-GSH analysis, equal number of participants (half of the participants) has been taken from each group expecting that the half of study population would represent the total.

Thus further studies for longer period in multiple locations in bigger As exposed population using locally produced or imported Se-enriched lentils is required to confirm our findings.

Chapter 5

CONCLUSIONS

The findings of this thesis convey the message that, for the people chronically exposed to higher levels of As, daily consumption of the RDA level of Se through lentils can be one of the possible ways to mitigate As related health hazards by increasing urinary As excretion, blood Se levels and offering modest health benefits. However, we did not observe any effect of the dietary RDA dose of Se supplementation on the levels of oxidative stress marker, antioxidants, inflammatory markers, and markers of hypertension (blood pressure) and cardiovascular diseases (plasma lipids). Whereas lentil supplementation, regardless Se concentration, provides somewhat beneficial responses demonstrated by decreasing lung inflammatory marker (FE_{NO}), blood pressure, and lipids during the 6-month intervention period with continuous consumption of As contaminated drinking water. A large-scale, longer-term study is desirable in Bangladesh or another As-affected geographical region, using either locally produced, or imported, Se-rich crops to further substantiate these findings. Concurrent Se supplementation to double the RDA would provide further insight into the potential benefits of Se against arsenicosis. Further studies would elucidate the mechanism through which dietary supplementation of Se may translate into improved health status.

Chapter 6

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APPENDIX

Consent Form

Adults

Protocol No. PR **14013**	version 3.0	Protocol No. PR **14013**

Protocol Title: Mitigating arsenic related health problems in Bangladesh by introducing high-selenium lentils into the everyday diet.

Principal Investigators: Dr. Rubhana Raqib and Dr Judit Smits

Organizations: icddr,b; University of Calgary

Purpose of the research

Chronic arsenic (As) poisoning is a worldwide public health problem. In Bangladesh, about 45 million people are exposed to high As in drinking water and food. Various adverse health effects result from chronic exposure to As. The most effective approach to reduce As toxicity is to switch to low As water supplies. However, it is not always feasible due to costs and other complications. Reducing levels of the ingested arsenic is another strategy that may mitigate arsenic poisoning.

Selenium (Se) is a trace element that is essential for our health and it can also counteract toxic effects of arsenic. Bangladeshi soil is deficient in Se and thus lentils grown in Bangladesh are low in Se. Lentils grown in Saskatchewan, Canada have a high Se content. The major aim of this study is to evaluate the health benefits of consuming these high-Se lentils by As-exposed families for decreasing As burden, reversal of As toxicity and increasing As excretion from the body.

Why invited to participate in the study

We will enrol a total of 80 families (about 400 participants) from your community in Shahrasti, Chandpur who are affected by As-contaminated ground water. We like to invite you and your family to participate in this 6-month study because you live in this area.

Methods and procedures

If you agree or allow your child/children to participate in this study, you may expect the followings:

1. About 400 participants (n=200 in each arm) will be enrolled in the study. Once families are recruited, they will be assigned to either the control (low-Se lentil) or the treatment group (high-Se lentil) by use of a computer-generated 'randomization method'. Your family will be assigned to get either the high-Se or the low-Se lentils. Nobody in the study team will know whether you have been given the high-Se or the low-Se lentils.
2. Every week, a Field Assistant (FA) will visit the families to provide weekly supplies of lentils. The families will be expected to consume 65 grams of lentils per person (a 65 g scoop will be provided for measurement), twice a day as soup.
3. Female village health workers (FVHW) will visit homes twice weekly to promote and assess compliance of intake of lentils.
4. The FA will ask you/your child some questions regarding your health status and whether you/your child have had any illness during the past 7 days during his/her weekly visits. This will take about 10 minutes.
5. Health professionals will assess your/your child's overall health status in the beginning and at the end of the study.
6. You/your child will be interviewed/examined at 4 different time points:

a) 1st visit at home: To obtain consent and give dates to come to the Field Office.
b) 2nd visit in Field Office, day 1: An initial interview will be taken to gather occupational, socio-demographic and behavioural information. Specimens of 10 ml blood (2 teaspoon full), 10 ml urine, hair (approximately 0.5 gm, 1 inch long will be cut from back of the head at nape of the neck, as close to the scalp as possible), and stool (about 4-5 gm) will be obtained in specific containers from you/your child (See Chart below).

   Lung inflammation will be assessed by use of a small machine. You/your child will be asked to take a deep breath through a plastic mouthpiece attached to the small machine. When the lungs are full, you/your child will blow out through the same mouthpiece. Reading will be obtained from the machine. Blood pressure will also be measured.
c) 3rd visit in Field Office, 3 mo: Blood (10 ml), urine, stool, lung inflammation and blood pressure assessment as above.
d) 4th visit in Field Office, 6 mo: Blood (10 ml), urine, stool, hair, lung inflammation and blood pressure assessment as above.

Chart for investigations	**Home**	**Field Office**		
	Visit 1	**Visit 2**	**Visit 3**	**Visit 4**
Study Days	**Day -15 to -1**	**Day 1**	**3 mo**	**6 mo**
Consent	X	-	-	-
Interview/Questionnaire	X	-	-	-
Health status		X	-	X
Blood pressure		X	X	X
Blood (2 teaspoon)/visit		X	X	X
Stool		X	X	X
Urine		X	X	X
Hair		X	-	X
Lung inflammation status		X	X	X

Risks and benefits

The risks associated with this study are minimal. Sterile disposable syringes and needles will be used for collection of blood. The insertion of the needle will cause a brief and temporary pain and there is a small risk of bruise. We will take all necessary precautions to avoid any complications. For the lung inflammation test, some people may experience temporary shortness of breath (very rare), but it will resolve spontaneously soon after the test.

There is no direct benefit for you/your child from this study except that since all participants will eat lentils daily which are very nutritious regardless of Selenium content there is likely to be a small health benefit. The results of this study might benefit society in general because it will help to understand whether a simple solution like consumption of high-Se lentil will help to reduce As-induced toxicity in the community. This information may be useful for establishing policies.

Privacy, anonymity and confidentiality

Names, personal information, all results of the laboratory tests will be kept confidential, under lock and key. Only the investigators of this study, ethics committee, sponsors, and any law-enforcing agency in the event of necessity would have an access to the information. We will provide you with the results of any tests performed on you/your child, and would be happy to answer your questions about the study.

Future use of information

We will carefully preserve your/your child samples (blood, urine, stool and hair) for future use if needed for the next 5 years. We may use the samples for some measurements e.g. toxic metals and their effects on health status. In that case we will maintain the confidentiality about your/your child's identity.

Right not to participate and withdraw
Participation in this study is voluntary. It is your decision whether you/your child will participate in this study or not. At any time of the study, even after being enrolled you are/ your child is free to discontinue participation. However, if you/your child want(s) to leave the study, please let the health care worker know.

Principle of compensation
There will be no monetary compensation except for transport cost for visits to the Field Office (500 Tk/visit/family).

Answering your questions/Contact persons
If you have any problem or further question you may also contact your health care worker at the Shahrasti Field Office of icddr,b or Dr. Rubhana Raqib, Tel: 9827068 or IRB Secretariat, Mr. M. A. Salam Khan, Phone No: 9886498 or PABX 8860523-32 Extension 3206.

If you agree to participate in our study, please indicate that by putting your signature or your left thumb impression at the specified space below:

Thank you for your cooperation

_____ _____
Signature or left thumb impression of Participant/Guardian Date

_____ _____
Signature or left thumb impression of the witness Date

_____ _____
Signature of the PI or his/her representative Date

সম্মতি পত্র
প্রাপ্তবয়স্ক

Protocol No. PR **14013**	version 3.0	Protocol No. PR **14013**

প্রটোকলের নামঃ **Mitigating arsenic related health problems in Bangladesh by introducing high-selenium lentils into the everyday diet.**

গবেষকের নামঃ ডঃ রুবহানা রাকিব এবং ডঃ জুডিত স্মিথস।

গবেষণা প্রতিষ্ঠানের নামঃ আ ই সি ডি ডি আর,বি ; ইউনিভার্সিটি অফ ক্যালগিরি।

গবেষণার উদ্দেশ্য

দীর্ঘকালস্থায়ী আর্সেনিক জনিত বিষক্রিয়া একটি বিশ্বব্যাপী জনস্বাস্থ্য সমস্যা। বাংলাদেশে প্রায় ৪৫ মিলিয়ন মানুষ আর্সেনিক বিষক্রিয়ায় আক্রান্ত যা আর্সেনিক যুক্ত পানি পান করার বা খাদ্যের কারনে হয়ে থাকে। আর্সেনিকের দীর্ঘমেয়াদি প্রভাবে স্বাস্থের উপর বিভিন্ন বিরূপ প্রতিক্রিয়া হয় । আর্সেনিক জনিত বিষাক্ততা কমাতে সবচেয়ে কার্যকর পন্থা হল আর্সেনিক মুক্ত পানি সরবরাহ করা । কিন্তু, ইহা একটি ব্যয়বহুল ও জটিল প্রক্রিয়া, তাই সব সময় এটা করা সম্ভবপর হয় না। আরেকটি কৌশল হল কোন ওষুধ গ্রহন করা বা খাদ্য খাওয়া যা আর্সেনিকের বিষক্রিয়া কমাতে পারে।

সেলেনিয়াম (Se) আমাদের স্বাস্থ্যের জন্য অপরিহার্য একটি পুষ্টি উপাদান যা আর্সেনিক এর বিষাক্ত প্রভাব কমাতে পারে। বাংলাদেশের মাটিতে Se এর ঘাটতি থাকায় এখানে উৎপন্ন মসূর ডালে Se এর পরিমান কম । কানাডার সাস্কেচুওান (Saskatchewan) প্রদেশে উৎপন্ন ডালে Se এর পরিমান বেশি থাকে। এই গবেষণার প্রধান উদ্দেশ্য হল, বেশি-Se যুক্ত ডাল খাওয়ার ফলে আর্সেনিক আক্রান্ত পরিবারের স্বাস্থ্যের উপর কোন প্রভাব পড়ে কি না তা দেখা, শরীরে আর্সেনিকের বিষাক্ততা হ্রাস পায় কি না এবং শরীর থেকে আর্সেনিক বের হয়ে যায় কি না তা নির্ণয় করা।

কেন গবেষণায় অংশগ্রহণের আমন্ত্রণ জানানো হচ্ছে

আমরা শাহরাস্তি, চাঁদপুর থেকে ৮০টি পরিবার (প্রায় ৪00 জন অংশগ্রহণকারী) অন্তর্ভুক্ত করব, যারা আর্সেনিক-দূষিত পানি (মাটির নিচের) পান করে। যেহেতু আপনি এই এলাকার অধিবাসি তাই আপনাকে এবং আপনার পরিবারকে ছয় মাস স্থায়ী এই গবেষণায় অংশগ্রহণের আমন্ত্রণ জানাচ্ছি।

গবেষণা পদ্ধতি

আপনি / আপনার সন্তান যদি এই গবেষণায় অংশগ্রহণ করতে সম্মত হন, তাহলে নিচের পদ্ধতিগুলি অনুসরণ করা হবে-

১) এই গবেষণায় প্রায় ৪০০ জন অংশগ্রহণকারীকে অন্তর্ভুক্ত করা হবে (প্রতি দলে ২০০ জন)। অংশগ্রহণকারী পরিবারকে কম্পিউটার পদ্ধতির মাধ্যমে দুই দলে বিভক্ত করা হবে – কন্ট্রোল দল (কম-Se যুক্ত ডাল) এবং চিকিৎসা দল (বেশি Se-যুক্ত ডাল)। আপনার পরিবার হয় বেশি-Se যুক্ত ডাল পাবে অথবা কম-Se যুক্ত ডাল পাবে। আপনার পরিবারকে কোন ডালটি দেয়া হয়েছে তা এই গবেষক দলের কেউ জানবে না।
২) প্রতি সপ্তাহে, একজন ফিল্ড অ্যাসিস্ট্যান্ট (FA) প্রত্যেক পরিবারকে ডাল সরবরাহ করবে। পরিবারের প্রতি ব্যাক্তিকে দিনে দুইবার ৬৫ গ্রাম পরিমান ডালের সূপ প্রতিবারে খেতে হবে (৬৫ গ্রামের চামচ পরিমাপের জন্য দেয়া হবে)।
৩) গ্রাম্য নারী স্বাস্থ্য কর্মীরা (FVHW) নিয়মিত ডাল খাওয়া হচ্ছে কি না তা মূল্যায়নের জন্য সপ্তাহে দুই বার ভিজিট করবে।
৪) FA আপনাকে/আপনার সন্তানকে ১৫-২০ মিনিট সময় নিয়ে আপনার/আপনার সন্তানের স্বাস্থ্যের অবস্থা সংক্রান্ত কিছু প্রশ্ন করবেন এবং গত ভিজিট থেকে ৭ দিন পর্যন্ত আপনার/আপনার সন্তানের কোনও অসুস্থতা ছিল কিনা তা জানতে চাইবে।
৫) স্বাস্থ্য গবেষকেরা গবেষণার শুরুতে এবং শেষে আপনার/আপনার সন্তানের সামগ্রিক স্বাস্থের অবস্থা মূল্যায়ন করবেন।

৬) আপনাকে / আপনার সন্তানকে ৪টি ধাপে অনুসরণ করা হবে/ইনটারভিউ নিবে -

ক) <u>১ম ভিজিট বাড়িতে :</u> আপনার সম্মতি নিবে এবং আপনাকে/আপনার সন্তানকে ফিল্ড অফিসে আসার জন্য তারিখ দিবে।

খ) <u>২য় ভিজিট ফিল্ড অফিসে, ১ম দিন :</u> প্রাথমিক সাক্ষাত্কারে আপনার পেশাগত, সামাজিক ও আচরণগত তথ্য নেয়া হবে। আপনার/আপনার সন্তানের কাছ থেকে ১০মিলি রক্ত (পূর্ণ ২ চা চামচ), ১০ মিলি প্রস্রাব, চুল (আনুমানিক 0.5 গ্রাম, ১ ইঞ্চি লম্বা মাথার চামড়ার যতটা সম্ভব কাছ থেকে কর্তন করা হবে), এবং মল (প্রায় ৪-৫ গ্রাম) নির্দিষ্ট পাত্রে নেয়া হবে (নিচের টেবিল দেখুন)।

একটি ছোট যন্ত্র ব্যবহার করে ফুসফুসের প্রদাহ মূল্যায়ন করা হবে। প্রত্যেক পরিবারের সদস্যকে ছোট মেশিনের সাথে সংযুক্ত একটি প্লাস্টিক মুখনলের মাধ্যমে গভীর নিঃশ্বাস নিতে বলা হবে। ফুসফুস পূর্ণ হলে, আপনি/আপনার সন্তান একই মুখপাত্রে জোরে দম ছেড়ে দিবেন। তারপর মেশিন থকে ফলাফল নেয়া হবে। আপনার/ আপনার সন্তানের রক্তচাপও দেখা হবে।

গ) <u>৩য় ভিজিট ফিল্ড অফিসে, ৩য় মাস:</u> রক্ত (১০মিলি), মূত্র, মল নেয়া হবে ও ফুসফুসের প্রদাহ দেখা হবে (পূর্বের মতো) ।

ঘ) <u>৪র্থ ভিজিট ফিল্ড অফিসে, ৬ষ্ঠ মাস:</u> রক্ত (১০মিলি), মূত্র, মল, চুল নেয়া হবে ও ফুসফুসের প্রদাহ দেখা হবে (পূর্বের মতো) ।

অনুসন্ধান তালিকা	**বাড়ি**	**ফিল্ড অফিস**		
	১ম ভিজিট	**২য় ভিজিট**	**৩য় ভিজিট**	**৪র্থ ভিজিট**

গবেষণা দিন	দিন -১৫ থেকে -১	১ম দিন	৩য় মাস	৬ষ্ঠ মাস
সম্মতি পত্র	X	-	-	-
সাক্ষাৎকার / প্রশ্নাবলী	X	-	-	-
স্বাস্থ্য অবস্থা		X	-	X
রক্তচাপ	-	X	X	X
রক্ত (২ চা চামচ) /সাক্ষাৎ		X	X	X
পায়খানা		X	X	X
প্রস্রাব		X	X	X
চুল		X	-	X
ফুসফুসের প্রদাহ অবস্থা		X	X	X

ঝুঁকি এবং উপকারিতা

এই গবেষণায় ঝুঁকি খুব সামান্য। রক্ত সংগ্রহের জন্য জীবাণুমুক্ত সিরিঞ্জ ও সূঁচ (ব্যবহার করার পর ফেলে দেওয়া হবে) ব্যবহার করা হবে। সুই প্রবেশের সময় হাতে অল্প ও অস্থায়ী ব্যথা হতে পারে এবং সামান্য কালশিটে দাগ হতে পারে। আমরা যে কোন জটিলতা এড়ানোর জন্য প্রয়োজনীয় সতর্কতা অবলম্বন করব। ফুসফুসের প্রদাহ পরীক্ষার পর, কিছু কিছু মানুষের অস্থায়ী শ্বাসকষ্ট (খুব বিরল) হতে পারে, কিন্তু এটি পরীক্ষার পর অনায়াসে খুব শীঘ্রই সমাধান হবে।

এই গবেষণা থেকে আপনি/আপনার সন্তান হয়তো সরাসরি কোন উপকার পাবেন না শুধুমাত্র এই যে যেহেতু পরিবারের সবাই ডাল খাবে যা খুবই পুষ্টিকর (সেলেনিয়াম তাতে বেশী থাকুক বা কম থাকুক), এতে সবারই স্বাস্থের কিছুটা হলেও উপকার হবে। এই গবেষণা থেকে প্রাপ্ত ফলাফল সাধারণভাবে সমাজের উপকারে আসতে পারে কারন বেশী-Se যুক্ত ডাল খাওয়ার মত একটি সহজ সমাধানের ফলে আর্সেনিক জনিত বিষাক্ততা কমবে কি না তা বুঝতে সাহায্য করবে । এই তথ্যটি নীতি প্রতিষ্ঠার ক্ষেত্রে সহায়ক হতে পারে।

গোপনীয়তা

নাম, ব্যক্তিগত তথ্য, ল্যাবরেটরি পরীক্ষা সব ফলাফল তালাবদ্ধ অবস্থায় গোপন রাখা হবে। শুধুমাত্র এই গবেষণার কর্মকর্তা, ইথিকস কমিটি, গবেষণার খরচ বহনকারী প্রতিষ্ঠান এবং আইন প্রয়োগকারী সংস্থা একান্ত প্রয়োজনে এই তথ্য ব্যবহার করতে পারবে। আপনি চাইলে আমরা আপনার/আপনার সন্তানের পরীক্ষা নিরীক্ষার ফলাফল আপনাকে প্রদান করব এবং আপনার কোন প্রশ্ন থাকলে খুশিমনে তার উত্তর দিব।

ভবিষ্যতে তথ্য ব্যবহার

আমরা আগামী ৫ বছরের জন্য আপনার/আপনার সন্তানের নমুনা সমুহ (রক্ত, মূত্র, মল এবং চুল) সাবধানে সংরক্ষণ করব যাতে প্রয়োজনে ভবিষ্যতে ব্যবহার করা যায়, যেমন- স্বাস্থ্যের উপর বিষাক্ত ধাতুর প্রভাব। সেই ক্ষেত্রে আমরা আপনার/আপনার সন্তানের পরিচয় গোপন রাখব।

অংশগ্রহণ না করা বা অংশগ্রহণের পর প্রত্যাহার করা

এই গবেষণায় অংশগ্রহণ করা সম্পূর্ণ স্বতঃস্ফুর্ত। আপনি/আপনার সন্তান এই গবেষণায় অংশগ্রহণ করবেন কি না, এটি পুরোপুরি আপনার সিদ্ধান্ত । এমনকি নাম নথিভুক্ত হওয়ার পরও আপনি/আপনার সন্তান যে কোনো সময় নিজের ইচ্ছানুযায়ী গবেষণা থেকে নিজেকে প্রত্যাহার করতে পারবেন। আপনি নিজেকে/আপনার সন্তানকে যদি গবেষণা থেকে প্রত্যাহার করাতে চান তাহলে দয়া করে আমাদের স্বাস্থ্য সেবা কর্মীকে জানাবেন।

ক্ষতিপূরণ

ফিল্ড অফিসে আসার জন্য পরিবহন খরচ বাবদ ৫০০ টাকা/ভিজিত/পরিবার ছাড়া অন্য কোন ক্ষতিপূরণ দেয়া হবে না।

আপনার প্রশ্নের উত্তর/ যোগাযোগ

যদি আপনার কোন সমস্যা বা কোন প্রশ্ন থাকে তবে আপনি আই. সি. ডি. ডি. আর,বি. এর শাহারাস্তি ফিল্ড অফিসের সাস্থকর্মীর সাথে অথবা ডঃ রুবহানা রাকিব, ফোনঃ ৯৮২৭০৬৮ অথবা আই. আর. বি. সচিব-এম এ সালাম খান, ফোনঃ ৯৮৮৬৪৯৮ অথবা পিএবিএক্স - ৮৮৬০৫২৩-৩২ এক্সটেনশন - ৩২০৬ এর সাথে যোগাযোগ করতে পারেন।

যদি আপনি এই গবেষণায় অংশগ্রহণ করতে রাজি থাকেন বা আপনার সন্তানকে অংশগ্রহণ করতে অনুমতি দেন তাহলে নিম্নে স্বাক্ষর করুন বা বৃদ্ধাঙ্গুলির ছাপ দিন। আপনার সাহায্যের জন্য আপনাকে একান্ত ধন্যবাদ।

অংশগ্রহণকারী /অভিবাবকের স্বাক্ষর বা বৃদ্ধাঙ্গুলির ছাপ _____তারিখ

_____ _____

সাক্ষীর স্বাক্ষর বা বৃদ্ধাঙ্গুলির ছাপ তারিখ

_____

পি.আই. বা প্রতিনিধির স্বাক্ষর তারিখ

Assent Form

Children

Protocol No. PR **14013**	version 3.0	Protocol No. PR **14013**

Protocol Title: Mitigating arsenic related health problems in Bangladesh by introducing high-selenium lentils into the everyday diet.

Principal Investigators: Dr. Rubhana Raqib and Dr Judit Smits

Organizations: icddr,b; University of Calgary

Purpose of the research

Chronic arsenic (As) poisoning is a worldwide public health problem. In Bangladesh, about 45 million people are exposed to high As in drinking water and food. Various adverse health effects result from chronic exposure to As. The most effective approach to reduce As toxicity is to switch to low As water supplies. However, it is not always feasible due to costs and other complications. Reducing levels of the ingested arsenic is another strategy that may mitigate arsenic poisoning.

Selenium (Se) is a trace element that is essential for our health and it can also counteract toxic effects of arsenic. Bangladeshi soil is deficient in Se and thus lentils grown in Bangladesh are low in Se. Lentils grown in Saskatchewan, Canada have high Se content. The major aim of this study is to evaluate the health benefits of consuming these high-Se lentils by As-exposed families for decreasing As burden, reversal of As toxicity and increasing As excretion from the body.

Why invited to participate in the study

We will enrol a total of 80 families (about 400 participants) from your community in Shahrasti, Chandpur who are affected by As-contaminated ground water. We like to invite you and your family to participate in this 6-month study because you live in this area.

Methods and procedures

If you agree to participate in this study, you may expect the followings:

7. About 400 participants (n=200 in each arm) will be enrolled in the study. Once families are recruited, they will be assigned to either the control (low-Se lentil) or the treatment group (high-Se lentil) by use of a computer-generated 'randomization method'. Your family will be assigned to get either the high-Se or the low-Se lentils. Nobody in the study team will know whether your family have been given the high-Se or the low-Se lentils.
8. Every week, a field assistant (FA) will visit the families to provide weekly supplies of lentils. The families will be expected to consume 65 grams of lentils per person (a 65 g scoop will be provided for measurement), twice a day as soup.
9. Female village health workers (FVHW) will visit homes twice weekly to promote and assess compliance of intake of lentils.
10. The FA will ask you some questions regarding your health status and whether you have had any illness during the past 7 days during his/her weekly visits. This will take about 15-20 minutes.
11. Health professionals will assess your overall health status in the beginning and at the end of the study.
12. You will be interviewed/examined at 4 different time points:

    e) 1st visit at home: To obtain consent and give dates to come to the Field Office.

f) 2nd visit in Field Office, day 1: An initial interview will be taken to gather educational, socio-demographic and behavioural information. Specimens of 10 ml blood (2 teaspoon full), 10 ml urine, hair (approximately 0.5 gm, 1 inch long will be cut from back of the head at nape of the neck, as close to the scalp as possible), and stool (about 4-5 gm) will be obtained from in specific containers from you.

Lung inflammation will be assessed by use of a small machine. You will be asked to take a deep breath through a plastic mouthpiece attached to the small machine. When the lungs are full, you will blow out through the same mouthpiece. Reading will be obtained from the machine. Your blood pressure will also be measured.

g) 3rd visit in Field Office, 3 mo: Blood (10 ml), urine, stool, lung inflammation, blood pressure assessment as above.

h) 4th visit in Field Office, 6 mo: Blood (10 ml), urine, stool, hair, lung inflammation, blood pressure assessment as above.

Chart for investigations	**Home**	**Field Office**		
	Visit 1	**Visit 2**	**Visit 3**	**Visit 4**
Study Days	**Day -15 to -1**	**Day 1**	**3 mo**	**6 mo**
Consent/Assent	X	-	-	-
Interview/Questionnaire	X	-	-	-
Health status		X	-	X
Bllod pressure		X	X	X
Blood (2 teaspoon)/visit		X	X	X
Stool		X	X	X
Urine		X	X	X
Hair		X	-	X
Lung inflammation status		X	X	X

Risks and benefits

The risks associated with this study are minimal. Sterile disposable syringes and needles will be used for collection of blood. The insertion of the needle will cause a brief and temporary pain and there is a small risk of bruise. We will take all necessary precautions to avoid any complications. For the lung inflammation test, some people may experience temporary shortness of breath (very rare), but it will resolve spontaneously soon after the test.

There is no direct benefit for you from this study except that since all participants will eat lentils daily which are very nutritious regardless of Selenium content there is likely to be a small health benefit. The results of this study might benefit society in general because it will help to understand whether a simple solution like consumption of high-Se lentil will help to reduce As-induced toxicity in the community. This information may be useful for establishing policies.

Privacy, anonymity and confidentiality

Names, personal information, all results of the laboratory tests will be kept confidential, under lock and key. Only the investigators of this study, ethics committee, sponsors, and any law-enforcing agency in the event of necessity would have an access to the information. We will provide you with the results of any tests performed on you, and would be happy to answer your questions about the study.

Future use of information

We will carefully preserve your samples (blood, urine, stool and hair) for future use if needed for the next 5 years. We may use the samples for some measurements e.g. toxic metals and their effects on health status. In that case we will maintain the confidentiality about your identity.

Right not to participate and withdraw
Participation in this study is voluntary. You may decide to participate or not in this study. At any time of the study, even after being enrolled you are free to discontinue participation. However, if you want to leave the study, please let the health care worker know.

Principle of compensation
There will be no monetary compensation except for transport cost for visits to the Field Office (500 Tk/visit/family).

Answering your questions/Contact persons
If you have any problem or further question you may also contact your health care worker at the Shahrasti Field Office of icddr,b or Dr. Rubhana Raqib, Tel: 9827068 or IRB Secretariat, Mr. M. A. Salam Khan, Phone No: 9886498 or PABX 8860523-32 Extension 3206.

If you agree to participate in our study, please indicate that by putting your signature or your left thumb impression at the specified space below:

Thank you for your cooperation

_____	_____
Signature or left thumb impression of child participant	Date
_____	_____
Signature or left thumb impression of Parent / Guardian	Date
_____	_____
Signature or left thumb impression of the witness	Date
_____	_____
Signature of the PI or his/her representative	Date

সম্মতি পত্র
শিশু

Protocol No. PR **14013**	version 3.0	Protocol No. PR **14013**

প্রটোকলের নামঃ **Mitigating arsenic related health problems in Bangladesh by introducing high-selenium lentils into the everyday diet.**

গবেষকের নামঃ ডঃ রুবহানা রাকিব এবং ডঃ জুডিত স্মিথস

গবেষণা প্রতিষ্ঠানের নামঃ আ ই সি ডি ডি আর,বি ; ইউনিভার্সিটি অফ ক্যালগিরি।

গবেষণার উদ্দেশ্য

দীর্ঘকালস্থায়ী আর্সেনিক জনিত বিষক্রিয়া একটি সাধারন জনস্বাস্থ্য সমস্যা। বাংলাদেশে প্রায় ৪৫ মিলিয়ন মানুষ আর্সেনিক বিষক্রিয়ায় আক্রান্ত যা আর্সেনিক যুক্ত পানি বা খাদ্যের কারনে হয়ে থাকে। আর্সেনিকের দীর্ঘমেয়াদি প্রভাবে শরীরে বিভিন্ন সমস্যা হয়। আর্সেনিকের বিষক্রিয়া কমাতে সবচেয়ে ভাল উপায় হল আর্সেনিক মুক্ত পানি সরবরাহ করা। কিন্তু, ইহা একটি ব্যয়বহুল ও জটিল প্রক্রিয়া। আরেকটি উপায় হতে পারে কোন ওষুধ দিয়ে আর্সেনিক বিষক্রিয়া কমানো।

সেলেনিয়াম (Se) আমাদের স্বাস্থ্যের জন্য একটি প্রয়োজনীয় পুষ্টি উপাদান যা আর্সেনিক এর বিষক্রিয়া কমাতে পারে। বাংলাদেশের মাটিতে Se কম থাকায় এখানে উৎপন্ন মসূর ডালে Se এর পরিমান কম। কানাডার সাস্কেচুওান (Saskatchewan) প্রদেশের ডালে Se এর পরিমান বেশি থাকে। আমরা তাই এই গবেষণায় দেখতে চাই যে বেশি Se-যুক্ত ডাল খাওয়ার ফলে আর্সেনিক আক্রান্ত পরিবারের স্বাস্থ্যের উপর কোন প্রভাব পড়ে কি না, শরীরে আর্সেনিকের বিষাক্ততা কমে কি না এবং শরীর থেকে আর্সেনিক বের হয়ে যায় কি না।

কেন গবেষণায় অংশগ্রহণের আমন্ত্রণ জানানো হচ্ছে

আমরা শাহরাস্তি, চাঁদপুর থেকে ৮০টি পরিবার (প্রায় ৪00 জন অংশগ্রহণকারীদের মধ্যে) অন্তর্ভুক্ত করব, যারা আর্সেনিক-দূষিত পানি (মাটির নিচের) দ্বারা আক্রান্ত (পান করে)। যেহেতু আপনি এই এলাকার অধিবাসি তাই আপনাকে এবং আপনার পরিবারকে ছয় মাস স্থায়ী এই গবেষণায় অংশগ্রহণের আমন্ত্রণ জানাচ্ছি।

গবেষণা পদ্ধতি

আপনি যদি এই গবেষণায় অংশগ্রহণ করতে সম্মত হন, তাহলে নিচের পদ্ধতিগুলি অনুসরণ করা হবে-

১) এই গবেষণায় প্রায় ৪০০ জন অংশগ্রহণকারীকে অন্তর্ভুক্ত করা হবে (প্রতি দলে ২০০ জন)। অংশগ্রহণকারী পরিবারকে কম্পিউটার পদ্ধতির মাধ্যমে দুই দলে বিভক্ত করা হবে – কন্ট্রোল দল (কম-Se যুক্ত ডাল) এবং চিকিৎসা দল (বেশি Se-যুক্ত ডাল)। একটি পরিবার কোন ডালটি পাবে

(বেশি-Se যুক্ত ডাল অথবা কম-Se যুক্ত ডাল) তা মুদ্রা নিক্ষেপণের (লটারি) মাধ্যমে নির্ধারণ করা হবে। আপনাকে বেশি-Se যুক্ত ডাল নাকি কম-Se যুক্ত ডাল দেয়া হয়েছে তা এই গবেষক দলের কেউ জানবে না।

২) প্রতি সপ্তাহে, একজন ফিল্ড অ্যাসিস্ট্যান্ট (FA) প্রত্যেক পরিবারকে ডাল সরবরাহ করবে। পরিবারের প্রতি ব্যাক্তিকে দিনে দুইবার ৬৫ গ্রাম পরিমান ডালের সূপ প্রতিবারে খেতে হবে (৬৫ গ্রামের চামচ পরিমাপের জন্য দেয়া হবে)।

৩) গ্রাম্য নারী স্বাস্থ্য কর্মীরা (FVHW) নিয়মিত ডাল খাওয়া হচ্ছে কি না তা মূল্যায়নের জন্য সপ্তাহে দুই বার ভিজিট করবে।

৪) FA আপনাকে ১৫-২০ মিনিট সময় নিয়ে আপনার স্বাস্থ্যের অবস্থা সংক্রান্ত কিছু প্রশ্ন করবেন এবং গত ভিজিট থেকে ৭ দিন পর্যন্ত আপনার কোনও অসুস্থতা ছিল কিনা তা জানতে চাইবে।

৫) স্বাস্থ্য গবেষক গবেষণার শুরুতে এবং শেষে আপনার সামগ্রিক স্বাস্থ্য অবস্থা মূল্যায়ন করবেন।

৬) আপনাকে ৪টি ধাপে অনুসরণ করা হবে/ইনটারভিউ নেওয়া হবে-

ক) <u>১ম ভিজিট বাড়িতে :</u> আপনার সম্মতি নিবে এবং আপনাকে ফিল্ড অফিসে আসার জন্য তারিখ দিবে।

খ) <u>২য় ভিজিট ফিল্ড অফিসে, ১ম দিন :</u> প্রাথমিক সাক্ষাত্কারে আপনার পড়াশুনা, সামাজিক ও আচরণগত তথ্য নেয়া হবে। আপনার কাছ থেকে ১০মিলি রক্ত (পূর্ণ ২ চা চামচ), ১০ মিলি প্রস্রাব, চুল (আনুমানিক 0.5 গ্রাম, ১ ইঞ্চি লম্বা মাথার চামড়ার যত সম্ভব কাছ থেকে কর্তন করা হবে), এবং মল (প্রায় ৪-৫ গ্রাম) নির্দিষ্ট পাত্রে নেয়া হবে।

একটি ছোট যন্ত্র ব্যবহার করে ফুসফুসের প্রদাহ মূল্যায়ন করা হবে। প্রত্যেক পরিবারের সদস্যকে ছোট মেশিনের সাথে সংযুক্ত একটি প্লাস্টিক মুখনলের মাধ্যমে গভীর নিঃশ্বাস নিতে বলা হবে। ফুসফুস পূর্ণ হলে, আপনি একই মুখপাত্রে জোরে দম ছেড়ে দিবেন। তারপর মেশিন থকে ফলাফল নেয়া হবে। আপনার রক্তচাপও দেখা হবে।

গ) <u>৩য় ভিজিট ফিল্ড অফিসে, ৩য় মাস:</u> রক্ত (১০মিলি), মূত্র, মল নেয়া হবে, ফুসফুসের প্রদাহ ও রক্তচাপ দেখা হবে (পূর্বের মতো)।

ঘ) <u>৪র্থ ভিজিট ফিল্ড অফিসে, ৬ষ্ঠ মাস:</u> রক্ত (১০মিলি), মূত্র, মল, চুল নেয়া হবে, ফুসফুসের প্রদাহ ও রক্তচাপ দেখা হবে (পূর্বের মতো)।

অনুসন্ধান তালিকা	**বাড়ি**	**ফিল্ড অফিস**		
	১ম ভিজিট	**২য় ভিজিট**	**৩য় ভিজিট**	**৪র্থ ভিজিট**
গবেষণা দিন	**দিন -১৫ থেকে -১**	**১ম দিন**	**৩য় মাস**	**৬ষ্ঠ মাস**
সম্মতি পত্র	X	-	-	-
সাক্ষাৎকার / প্রশ্নাবলী	X	-	-	-

স্বাস্থ্য অবস্থা		X	-	X
রক্তচাপ	-	X	X	X
রক্ত (২ চা চামচ) /সাক্ষাৎ		X	X	X
পায়খানা		X	X	X
প্রস্রাব		X	X	X
চুল		X	-	X
ফুসফুসের প্রদাহ অবস্থা		X	X	X

ঝুঁকি এবং উপকারিতা

এই গবেষণায় ঝুঁকি খুব সামান্য। রক্ত সংগ্রহের জন্য জীবাণুমুক্ত সিরিঞ্জ ও সূঁচ (ব্যবহার করার পর ফেলে দেওয়া হবে) ব্যবহার করা হবে। সুই প্রবেশের সময় হাতে অল্প ও অস্থায়ী ব্যথা হতে পারে এবং সামান্য কালশিটে দাগ হতে পারে। আমরা যে কোন জটিলতা এড়ানোর জন্য প্রয়োজনীয় সতর্কতা অবলম্বন করব। ফুসফুসের প্রদাহ পরীক্ষার পর, কিছু কিছু মানুষের অস্থায়ী শ্বাসকষ্ট (খুব বিরল) হতে পারে, কিন্তু এটি পরীক্ষার পর অনায়াসে খুব শীঘ্রই সমাধান হবে।

এই গবেষণা থেকে আপনি হয়তো সরাসরি কোন উপকার পাবেন না শুধুমাত্র এই যে যেহেতু পরিবারের সবাই ডাল খাবে যা খুবই পুষ্টিকর (সেলেনিয়াম তাতে বেশী থাকুক বা কম থাকুক), এতে সবারই স্বাস্থের কিছুটা হলেও উপকার হবে। এই গবেষণা থেকে প্রাপ্ত ফলাফল সাধারণভাবে সমাজের উপকারে আসতে পারে কারন বেশী-Se যুক্ত ডাল খাওয়ার মত একটি সহজ সমাধানের ফলে আর্সেনিক জনিত বিষাক্ততা কমবে কি না তা বুঝতে সাহায্য করবে । এই তথ্যটি নীতি প্রতিষ্ঠার ক্ষেত্রে সহায়ক হতে পারে।

গোপনীয়তা

নাম, ব্যক্তিগত তথ্য, ল্যাবরেটরি পরীক্ষা সব ফলাফল তালাবদ্ধ অবস্থায় গোপন রাখা হবে। শুধুমাত্র এই গবেষণার কর্মকর্তা, ইথিকস কমিটি, খরচ বহনকারী প্রতিষ্ঠান এবং আইন প্রয়োগকারী সংস্থা একান্ত প্রয়োজনে এই তথ্য ব্যবহার করতে পারবে। আপনি চাইলে আমরা আপনার পরীক্ষা নিরীক্ষার ফলাফল আপনাকে প্রদান করব এবং আপনার কোন প্রশ্ন থাকলে খুশিমনে তার উত্তর দিব।

ভবিষ্যতে তথ্য ব্যবহার

আমরা আগামী ৫ বছরের জন্য আপনার নমুনাসমুহ (রক্ত, মূত্র, মল এবং চুল) সাবধানে সংরক্ষণ করবে যাতে প্রয়োজনে ভবিষ্যতে ব্যবহার করা যায়, যেমন- স্বাস্থ্যের উপর বিষাক্ত ধাতুর প্রভাব। সেই ক্ষেত্রে আমরা আপনার পরিচয় গোপন রাখব।

অংশগ্রহণ না করা বা অংশগ্রহণের পর প্রত্যাহার করা

এই গবেষণায় অংশগ্রহণ করা সম্পূর্ণ স্বতঃস্ফুর্ত। আপনি এই গবেষণায় অংশগ্রহণে করবেন কি না , এটি আপনার সিদ্ধান্ত । এমনকি নাম নথিভুক্ত হওয়ার পরও আপনি যে কোনো সময় নিজের ইচ্ছানুযায়ী গবেষণা থেকে নিজেকে প্রত্যাহার করতে পারবেন। আপনি যদি গবেষণা থেকে নিজেকে প্রত্যাহার করতে চান তাহলে দয়া করে আমাদের স্বাস্থ্য সেবা কর্মীকে জানান।

ক্ষতিপূরণ

ফিল্ড অফিসে আসার জন্য পরিবহন খরচ বাবদ ৫০০ টাকা/ভিজিত/পরিবার ছাড়া অন্য কোন ক্ষতিপূরণ দেয়া হবে না।

আপনার প্রশ্নের উত্তর/ যোগাযোগ

যদি আপনার কোন সমস্যা বা কোন প্রশ্ন থাকে তবে আপনি আ ই সি ডি ডি আর,বি এর শাহারাস্তি ফিল্ড অফিসের সাস্থকর্মীর সাথে অথবা ডঃ রুবহানা রাকিব, ফোনঃ ৯৮২৭০৬৮ অথবা আই আর বি সচিব-এম এ সালাম খান, ফোনঃ ৯৮৮৬৪৯৮ অথবা পিএবিএক্স - ৮৮৬০৫২৩-৩২ এক্সটেনশন - ৩২০৬**.** এর সাথে যোগাযোগ করতে পারেন।

যদি আপনি এই গবেষণায় অংশগ্রহণ করতে রাজি থাকেন তাহলে নিম্নে স্বাক্ষর করুন বা বৃদ্ধাঙ্গুলির ছাপ দিন। আপনার সাহায্যের জন্য আপনাকে একান্ত ধন্যবাদ।

_____ _____

অংশগ্রহণকারী শিশুর স্বাক্ষর বা বৃদ্ধাঙ্গুলির ছাপ
তারিখ

_____ _____

অংশগ্রহণকারীর অভিবাবকের স্বাক্ষর বা বৃদ্ধাঙ্গুলির ছাপ
তারিখ

_____ _____

সাক্ষীর স্বাক্ষর বা বৃদ্ধাঙ্গুলির ছাপ তারিখ

_____ _____

পি.আই. বা প্রতিনিধির স্বাক্ষর তারিখ

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প্রাথমিক প্রশ্নাবলী

Number: PR-14013

Study Title: Mitigation Arsenic Related Health Problems in Bangladesh by Introducing High-Selenium Lentils Into Everyday Diet

Households ID: ☐☐☐☐

১. অংশগ্রহনকারীর নাম:

.....

২. অংশগ্রহনকারীর মোবাইল নং .....

বাড়ীর প্রধানের এর নাম ..... মোবাইল নং ..... বাড়ীর নাম.....

গ্রাম..... ইউনিয়ন.....

অন্যান্য .....

৩. আপনার বাড়ীর মোট সদস্য সংখ্যা (আপনি নিজে সহ) .....

সদস্যদের তথ্য:

সদস্য নং	নাম	লিঙ্গ ১- পুরুষ ২-মহিলা	বয়স	বাড়ীর প্রধান এর সাথে সম্পর্ক	বৈবাহিক অবস্থা	গর্ভাবস্থা	পেশা	শিক্ষাগত যোগ্যতা
০১								
০২								
০৩								
০৪								
০৫								
০৬								
০৭								
০৮								
০৯								
১০								

সদস্যদের তথ্যসমূহের কোড:

বৈবাহিক অবস্থা	নারীর গর্ভাবস্থা
১. বিবাহিত	১. গর্ভবতী
২. তালাক প্রাপ্ত	২. গর্ভবতী না
৩. পৃথকীকৃত/আলাদা	৮৮. অজানা
৪. অবিবাহিত	
শিক্ষাগত যোগ্যতা	
১. অশিক্ষিত	৫. এস.এস.সি/দাখিল
২. শুধুমাত্র স্বাক্ষর করতে পারেন	৬. এইচ.এস.সি/আলিম
৩. ১ম শ্রেণী- ৫ম শ্রেণী	৭. স্নাতক/ডিপ্লোমা

৪. ১০ম শ্রেণীর নিচে		
বাড়ীর প্রধান এর সাথে সম্পর্ক	পেশা	
১. পরিবার প্রধান	১. বেকার	৯. শিক্ষক
২. পরিবার প্রধান এর স্ত্রী	২. গৃহিনী	১০. ছাত্র
৩. ছেলে বা মেয়ে/ পালিত ছেলে বা মেয়ে	৩. বাসার কাজে সাহায্যকারী	১১. ডাক্তার
৪. ভাই বা বোন/ শালা বা শ্যালিকা	৪. রিকশা / ভ্যান / ট্রলি চালক	১২. ব্যবসা
৫. পিতা বা মাতা/ শশুর বা শাশুরি	৫. দিন মজুর/ শ্রমিক	৭৭. অন্যান্য
৬. নাতি/নাত্নি	৬. কৃষক / জেলে	
৭. দাদা/দাদি	৭. দর্জি / নাপিত / কারিগর	
৮. গৃহস্থালি কাজে সাহায্যকারী	৮. চাকুরী	

আর্থ সামাজিক অবস্থা			
০১.	আপনি কতদিন যাবত এই বাসায় বসবাস করছেন? .....বছর		
০২.	বাড়ির মালিকানা	নিজে মালিক	১
		ভাড়া	২
		চাকুরীসুত্রে পাওয়া	৩
০৩	বাড়ী কি দিয়ে তৈরি?	মাটি (কাঁচা)	১
		পাটকাঠীটি/ছন	২
		বাঁশ/কাঠ	৩
		টিন	৪
		আংশিক পাকা	৫
		পাকা (ইট/সিমেন্ট)	৬
		অন্যান্য (উল্লেখ করুন)	৭৭
০৪.	মাসিক গড় ব্যয়	খাওয়া বাবদ -.....টাকা	
		পরিবহন বাবদ-.....টাকা	
		শিক্ষা বাবদ- .....টাকা	
		অনন্যা .....টাকা	
		মোট ব্যয় .....টাকা	
স্বাস্থ্যব্যবস্থা			
০৫	বাড়িতে কি হাত ধোয়ার সাবান ব্যবহার করেন?	□হ্যাঁ	১
		□না	২
০৬	বর্জ্য/ আবর্জনা ফেলার জায়গা	নির্দিষ্ট জায়গা	১
		অনির্দিষ্ট জায়গা	২
০৭	আপনার বাসায় কি ধরনের পয়ঃনিষ্কাশন ব্যবস্থা আছে?	আধুনিক পাকা ল্যাট্রিন	১
		পিট ল্যাট্রিন- পানি জমা থাকে	২
		পিট ল্যাট্রিন- পানি জমা থাকে না	৩
		কাঁচা বা ঝুলন্ত পায়খানা	৪
		খোলা জায়গা	৫
		অন্যান্য (উল্লেখ করুন).	৭৭
	খাওয়ার পানি		
০৮	আপনার খাওয়ার পানির উৎস কি?	নলকূপ	১
		গভীর নলকূপ	২
		কুয়া	৩
		পুকুরের পানি	৪
		সাপ্লাই পানি	৫

		নদীর পানি	৬
		অন্যান্য (উল্লেখ করুন).....	৭৭
৯	আপনি পানি পরিশোধন করেন কিনা?	☐হ্যাঁ	১
		☐না	২
১০	আপনি কি ধরনের পানি পান করেন?	ফুটানো	১
		ফিল্টার	২
		রাসায়নিকভাবে বিশুদ্ধ	৩
		বিশুদ্ধ না করে	৪
		অন্যান্য (উল্লেখ করুন).....	৭৭
		জানিনা	৮৮
১১	আপনার বাড়ী থেকে পানির উৎসের দূরত্ব? ....মি/গজ		
	আর্সেনিকে আক্রান্ত		
১২	আপনি কি জানেন যে আপনি/আপনার পরিবার আর্সেনিক আক্রান্ত কি না?	☐হ্যাঁ	১
		☐না	২
	যদি উত্তর হ্যাঁ হয়, তাহলে কতদিন যাবত.....		
১৩	আর্সেনিক আক্রান্ত হওয়ার কয়েক বছরের মধ্যে আপনি/আপনার সন্তানের কোন শারীরিক অস্বাভাবিকতা লক্ষ্য করেছেন কি?	☐হ্যাঁ	১
		☐না	২
১৪	কি ধরনের শারীরিক সমস্যা (উল্লেখ করুণ):		

সাক্ষাৎকার গ্রহণের অবস্থা.....পরিপূর্ণ.....আংশিক.....

সম্মতি প্রত্যাহার.....এলাকাত্যাগ.....

সাক্ষাৎকার গ্রহনকারীর নাম.....

তারিখ.....সময় ☐☐ : ☐☐ AM/PM

সাক্ষাৎকার গ্রহনকারীর স্বাক্ষর.....

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Number: PR-14013

Study Title: Mitigation Arsenic Related Health Problems in Bangladesh by Introducing High-Selenium Lentils into Everyday Diet

বাড়ীর **ID:** ☐☐ পরিবারের **ID:** ☐☐
তারিখ.....

অংশগ্রহনকারীর (মা/যে রান্না করে) নাম:.....

নং	ডাল খাওয়ার নিয়ম যাচাই		
১	আপনি গত সপ্তাহে প্রতিদিন নির্দেশনা অনুসারে ডাল খেয়েছিলেন?	☐হ্যাঁ	☐না
২	যদি না খেয়ে থাকেন, তাহলে কতদিন খান নাই?		
৩	গতকাল আপনি কতবার ডাল খেয়েছিলেন? (দিনে একবার=১, দিনে দুইবার= ২/৩ বার) (যে কোন প্রাপ্তবয়স্ক অথবা শিশুকে জিগ্যেস করতে হবে)	☐১	☐২ ☐৩
৪	প্রতিদিন ডাল রান্নায় আপনি কি কোনো অসুবিধা বোধ করে ছিলেন? (মাকে/যে রান্না করে তাকে জিজ্ঞেস করুন) যদি হ্যাঁ হয়, তাহলে কি ধরেণের অসুবিধা (উল্লেখ করুণ):	☐হ্যাঁ	☐না
৫	আপনি দিনে কতবার ডাল রান্না করেছেন? (দিনে ১ বার=১, দিনে ২ বার=২)	☐১	☐২
৬	আপনি কি ডাল পরিমাপের জন্য আমদের দেয়া পাত্র-চামচ ব্যাবহার করেছেন?	☐হ্যাঁ	☐না
	যদি না হয়, তাহলে কিভাবে পরিমাপ করেন (উল্লেখ করুণ): ..... যদি হ্যাঁ হয়, তাহলে কয় পাত্র-চামচ ডাল রান্না করেছেন? .....।		
৭	গত সপ্তাহে কোন দিন কি পাত্র-চামচ ব্যাবহার করতে ভুলে গিয়েছিলেন? যদি হ্যাঁ হয়, তাহলে কতদিন	☐হ্যাঁ	☐না
৮	আমাদের দেয়া ডালের স্বাদ ভাল ছিল ?	☐হ্যাঁ	☐না
৯	আপনি কি রান্না করা ডাল প্রত্যেক সদস্যকে সমান ভাবে খেতে দিয়েছেন?	☐হ্যাঁ	☐না
১০	বড়রা কি রান্না করা ডাল গবেষণার অন্তর্ভুক্ত ছোটদের চেয়ে বেশি খেয়েছে?	☐হ্যাঁ	☐না
	যদি হ্যাঁ হয়, তাহলে প্রত্যেক সদস্য কয় পাত্র/কি পরিমান করে ডাল খেয়েছেন ? প্রাপ্তবয়স্ক #১..... প্রাপ্তবয়স্ক#২.....প্রাপ্তবয়স্ক#৩.....প্রাপ্তবয়স্ক#৪.....		

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	শিশু#১.....শিশু#২.....শিশু#৩..... শিশু#৪.....		
১১	আপনি কি কারও সাথে ডাল ভাগাভাগি করে খেয়েছেন, যে এই গবেষণার অন্তর্ভুক্ত নয়? (উদাঃ মেহমান) যদি হ্যাঁ হয়, তাহলে পরিমান উল্লেখ করুন	☐হ্যাঁ	☐না
১২	আপনি কি মেহমানের জন্য অতিরিক্ত ডাল রান্না করেছিলেন? যদি হ্যাঁ হয়, তাহলে পরিমান উল্লেখ করুন.....।	☐হ্যাঁ	☐না
১৩	গত সপ্তাহে নির্ধারিত সময়ের পূর্বেই কি ডাল শেষ হয়ে গিয়েছিল?	☐হ্যাঁ	☐না
১৪	গত সপ্তাহে আপনার পরিবারের কোন সদস্য বাড়ীর বাইরে ছিল? যদি হ্যাঁ হয়, তাহলে কত দিন.....? বাড়ীর বাইরে খাবারের সংখ্যা.....	☐হ্যাঁ	☐না
১৫	গবেষণা কর্মী কি আপনার জন্য বরাদ্দকৃত ডাল সরবরাহ করেছিলেন?	☐হ্যাঁ	☐না
১৬	সপ্তাহ শেষে কি কোনও ডাল অবশিষ্ট আছে?যদি থাকে তহলে তা কি পরিমান (কয় পাত্র-চামচ) ?	☐হ্যাঁ	☐না

সাক্ষাৎকার গ্রহণের অবস্থা.....পরিপূর্ণ.....আংশিক.....

সম্মতি প্রত্যাহার..... এলাকাত্যাগ....

নারী স্বাস্থ্য কর্মীর নাম.....সময় ☐☐ : ☐☐

স্বাক্ষর..... তারিখ.....

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Number: PR-14013

Study Title: Mitigation Arsenic Related Health Problems in Bangladesh by Introducing High-Selenium Lentils into Everyday Diet

রোগ / অসুস্থতা

Study ID: ☐☐☐☐☐☐

তারিখ.....

অংশগ্রহনকারীর নাম:

.....

০১	আপনি কি কখনও হাঁপানিতে ভুগেছিলেন? যদি হ্যাঁ হয়, তাহলে (উল্লেখ করুণ) : সময়:	☐হ্যাঁ	☐না
০২	আপনার কি কখনও চর্মরোগ হয়েছিল? যদি হ্যাঁ হয়, তাহলে (উল্লেখ করুণ) : সময়:	☐হ্যাঁ	☐না
০৩	আপনার কোন এলার্জি সমস্যা আছে? যদি হ্যাঁ হয়, তাহলে (উল্লেখ করুণ) : সময়:	☐হ্যাঁ	☐না
০৪	আপনি কি কখনও জন্ডিসে ভুগেছিলেন? (চোখ, প্রস্রাব, ত্বক হলুদ হওয়া) যদি হ্যাঁ হয়, তাহলে (উল্লেখ করুণ) : সময়:	☐হ্যাঁ	☐না
০৫	আপনি গত ২ সপ্তাহের মধ্যে ঠাণ্ডা ও কাশি অথবা শ্বাসপ্রশ্বাস জনিত কোন সমস্যায় ভুগেছিলেন?যদি হ্যাঁ হয়, তাহলে (উল্লেখ করুণ) : সময়:	☐হ্যাঁ	☐না
০৬	আপনি গত ২ সপ্তাহের মধ্যে বুকে ব্যাথা সহ ঠাণ্ডা ও কাশি অথবা শ্বাসপ্রশ্বাস জনিত কোন সমস্যায় ভুগেছিলেন?	☐হ্যাঁ	☐না
০৭	আপনি কি এখনও এই ধরনের সমস্যায় ভুগছেন?	☐হ্যাঁ	☐না
০৮	আপনার কি গত ২ সপ্তাহের প্রসাবের সময় ব্যাথা/জ্বালা পোড়া বা অন্য কোন ধরনের অসুবিধা হয়েছিল? যদি হ্যাঁ হয়, তাহলে কতবার হয়েছিল.....	☐হ্যাঁ	☐না
০৯	যখন আপনার প্রসাবের সমস্যা হয়েছিল তখন কি আপনার জ্বর ছিল?	☐হ্যাঁ	☐না
১০	আপনার কি এখনও প্রসাবে সমস্যা আছে ?	☐হ্যাঁ	☐না
১১	আপনি গত ২ সপ্তাহের মধ্যে ডায়রিয়া জনিত সমস্যায় ভুগেছিলেন? যদি হ্যাঁ হয়, তাহলে দিনে কতবার পাতলা পায়খানা হয়েছিল এবং কতদিন	☐হ্যাঁ	☐না

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	ভুগেছিলেন?.....বার.....দিন।		
১২	আপনি কি পাতলা পায়খানার সময় রক্তের উপস্থিতি লক্ষ্য করেছেন? যদি হ্যাঁ হয়, তাহলে দিনে কতবার রক্ত পায়খানা হয়েছিল এবং কতদিন ভুগেছিলেন?.....বার.....দিন।	☐হ্যাঁ	☐না
১৩	আপনি কি এখনও ডায়রিয়া জনিত সমস্যায় ভুগছেন?	☐হ্যাঁ	☐না
১৪	গত ২ সপ্তাহের মধ্যে কি আপনার জ্বর হয়েছিল?	☐হ্যাঁ	☐না
	যদি হ্যাঁ হয়, তাহলে কতদিন ভুগেছিলেন		
১৫	আপনি কি গত ২ সপ্তাহে কোন স্বাস্থ্যসেবা বা চিকিৎসা নিয়েছিলেন ?	☐হ্যাঁ	☐না
১৬	সাস্থসেবার জন্য কোথায়/কার কাছে গিয়েছিলেন?	সরকারি হাসপাতাল	১
		ক্লিনিক	২
		ডাক্তার	৩
		ফার্মেসি	৪
		হাতুড়ে ডাক্তার	৫
১৭	কি সমস্যার জন্য আপনি চিকিৎসা নিয়েছেন?		

সাক্ষাৎকার গ্রহণের অবস্থা.....পরিপূর্ণ.....আংশিক.....

সম্মতি প্রত্যাহার.....এলাকাত্যাগ.....

সাক্ষাৎকার গ্রহনকারীর নাম.....সময় ☐☐ : ☐☐ AM/PM

সাক্ষাৎকার গ্রহনকারীর স্বাক্ষর.....

Department of Biochemistry and Molecular Biology
University of Dhaka
Dhaka –1000
Bangladesh

প্রাণরসায়ন ও অণুপ্রাণ বিজ্ঞান বিভাগ
ঢাকা বিশ্ববিদ্যালয়
ঢাকা - ১০০০
বাংলাদেশ

OFFICE OF ETHICAL REVIEW COMMITTEE
Department of Biochemistry and Molecular Biology
University of Dhaka, Bangladesh

No. BMBDU-ERC/EC/18/17 January 7, 2018

Ms. Evana Akhtar
Ph.D.Student
Department of Biochemistry and Molecular Biology
University of Dhaka, Dhaka-1000

Subject: Institutional Ethical Review Committee (ERC) Clearance

Dear Ms. Evana Akhtar,

With reference to your application on the above mentioned subject, this is to inform you that your Ph.D. Research Proposal titled "**Impact of introducing high-selenium lentils into the everyday diet for mitigating arsenic related health problems in Bangladesh"** has been reviewed and approved by the Institutional Ethical Review Committee (IERC) of the Department of Biochemistry and Molecular Biology, University of Dhaka in its 11th meeting held on January 6, 2018.

You are requested to follow the Institutional Ethical Review Committee (IERC) guidelines. Please note that failure to comply with the conditions of approval may result in withdrawal of ethical clearance approval of the proposal.

Prof. Dr. Yearul Kabir
Chairman
Ethical Review Committee
Department of Biochemistry and Molecular Biology
University of Dhaka, Dhaka-1000

Telephone: 9661920-73 Ext.7637 Fax: 880-2-8615583 Internet: http://www.bmbudhaka.net

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Memorandum

11 June 2014

To: Dr Rubhana Raqib
Principal Investigator of research protocol # PR-14013
Centre for Vaccine Sciences (CVS)

From: Professor K Z Mamun
Chairman
Ethical Review Committee (ERC)

Sub: Approval of research protocol # PR-14013

Approval Date: 11 June 2014
Expiration Date: 10 June 2015
Review Type: Full Committee Review
Risk Level: No more than minimal
Project type: New Project

Thank you for your memo dated 15 May 2014 attaching the modified version of your research protocol # PR-14013 entitled "Mitigating arsenic related health problems in Bangladesh by introducing high-selenium lentils into the everyday diet" addressing the issues raised by the committee in its March meeting held on 5 April 2014, at 3:00 pm, at the former PHSD Conference Room, 2nd floor of the Library Building to the satisfaction of the Committee. I am pleased to inform you that your protocol is **approved**. You will be required to observe the following terms and conditions in implementing the research protocol:

1. The research protocol is approved for 12-month period from the date of approval of the protocol by the Ethical Review Committee. The Federal regulations require review of an approved study not less than once per 12-month period. To comply with federal regulations, a continuing review application must be submitted to the IRB Secretariat for this study to continue beyond 10 June 2015.

   All necessary materials for continuing review must be reviewed with sufficient time for review and issuing continued approval before the expiration date. Failure to initiate a continuing review application in a timely fashion may result in discontinuation of study activities until approval can be renewed. Performing study activities, including data analysis, beyond the expiration date results in noncompliance of federal regulations.

2. The ERC approval shall automatically be revoked after one year if the protocol is not started. After one year, you shall have to seek approval for revalidation of the protocol by the ERC before starting.

GPO Box 128, Dhaka 1000, Bangladesh.
For Packages : 68, Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212, Bangladesh.
Phone : 880-2-9827001-10, Web : http://www.icddrb.org, Fax : 880-2-9827077

3. You should notify the IRB Secretariat of the start date of the protocol for updating in the integrated Navision system. The protocol start date will not be updated in the Navision system until receiving information from you. Therefore you will not be able to operate budget code and continue spending funds under the research protocol.

4. As Principal Investigator, the ultimate responsibility for scientific and ethical conduct including the protection of the rights and welfare of study participants vest upon you. You shall also be responsible for ensuring competence, integrity and ethical conduct of other investigators and staff directly involved in this research protocol.

5. You shall conduct the study in accordance with the ERC-approved protocol and shall fully comply with any subsequent determinations by the ERC.

6. You shall obtain prior approval from the Research Review Committee and the ERC for any modification in the approved research protocol and/or approved consent form(s), except in case of emergency to safeguard/ eliminate apparent immediate hazards to study participants. Such changes must immediately be reported to the ERC Chairman.

7. You shall recruit/enrol participants for this study strictly adhering to the criteria mentioned in the research protocol.

8. You shall obtain legally effective informed consent (i.e. consent should be free from coercion or undue influence) from the selected study participants or their legally responsible representative, as approved in the protocol, using the approved consent form prior to their enrolment in this study. Before obtaining consent, all prospective study participants must be adequately informed about the purpose(s) of the study, its methods and procedures, and also what would be done if they agree and also if they do not agree to participate in the study.

9. They must be informed that their participation in the study is voluntary and that they can withdraw their participation any time without any prejudice. Signed consent forms should be preserved for a period of at least five years following official termination of the study.

10. You shall promptly report the occurrence of any Serious Adverse Event or unanticipated problems of potential risk to study participants or others to the ERC in writing within 24 hours of such occurrences.

11. Any significant new findings, developing during the course of this study that might affect the risks and benefits and thus influence either participation in the study or continuation of participation should be reported in writing to the participants and the ERC.

12. You shall report progress of research to the ERC for continuing review of the implementation of the research protocol as stipulated in the ERC Guidelines. Relevant excerpt of ERC Guidelines and '*Annual/Completion* Report for Research Protocol involving Human Subjects' are attached for your information and guidance.

13. Data and/or samples should be collected and interviews should be conducted, as specified in the ERC-approved protocol, and confidentiality must be maintained. Data/samples must be protected by reasonable security,

safeguarding against risks such as their loss or unauthorized access, destructions, used by others, and modification or disclosure of data. Data/samples should not be disclosed, made available to or use for purposes other than those specified in the protocol, and shall be preserved for a period, as specified under Centre's policies/practices.

14. You shall promptly and fully comply with the decision of the ERC to suspend or withdraw its approval for the research protocol.

15. The ERC should be immediately notified if the protocol is discontinued before the expected date of completion.

Approved documents:

a. Protocol version no. 1.0
b. English and Bangla CF for head of the family
c. English and Bangla CF for adults
d. English and Bangla assent form for children
e. English and Bangla questionnaire

The IRB of icddr,b shall take into account the regulations of the Bangladesh Medical Research Council (BMRC), WHO, international guidelines for biomedical research as laid down by the Council of International Organization of Medical Sciences (CIOMS), the Declaration of Helsinki in relation to biomedical research involving human participants, ICH Guidelines on Good Clinical Practice (GCP), National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), and Division of Microbiology and Infectious Diseases (DMID). If there is any new declaration involving human participants, contents of such declaration should be appropriately adhered to and the applicable laws and policies of the local government.

The IRB of icddr,b is comprising of 17 members. According to the IRB SOP, at least two-third majority is mandatory to make the quorum. The members who attended in the above mentioned meeting where your proposal was discussed are listed below:

Sl. #	Name of the Members	Membership Status & discipline	Gender
1	Professor Kazi Zulfiquer Mamun	Virology/ Microbiology Chairperson	Male
2	Professor Meerjady Sabrina Flora	Epidemiology and Biostatistics Co-Chairperson	Female
3	Professor Mamunar Rashid	Nutrition	Male
4	Advocate U.M. Habibun Nessa Habiba	Law, Human Rights and Women Affairs	Female
5	Professor Jalaluddin Ashraful Haq	Microbiology	Male
6	Professor Chowdhury Ali Kawser	Paediatrics	Male

Sl. #	Name of the Members	Membership Status & discipline	Gender
7	Dr. Nafisa Lira Huq	Reproductive Health	Female
8	Professor Muhammad Abdur Rashid	Theology	Male
9	Professor Kamruzzaman Mozumder	Psychology	Male
10	Dr. Rubhana Raqib	Nutrition/Immunology	Female
11	Dr. Md. Nurul Alam	Demography and Epidemiology	Male
12	Professor Nazma Haque	Pharmacology	Female
13	Ms Shila Rani Hira	Nursing	Female
14	Professor Saria Tasnim	Obstetrician & Gynaecologist	Female

Dr Rubhana Raqib, Principal Investigator of the research protocol is a member of the ERC and did not participate in the voting for approval of the study.

I wish you success in running the above-mentioned study.

Cc: Director, CVS