Effect of Zinc Supplementation on Blood Glucose Level in Type 2 Diabetics: A Placebo Controlled Clinical Trial

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Certification

The thesis entitled "**Effect of Zinc Supplementation on Blood Glucose level in Type 2 Diabetics: A Placebo Controlled Clinical Trial"** has been completed sincerely and satisfactorily by Shayla Nasrin of registration no: 55, session 2015-2016, enrolled in the University of Dhaka, Bangladesh, for the degree of Doctor of Philosophy (PhD) in Nutrition and Food Science, is an original research work and record, and was supervised by us can be submitted to the examination committee for evaluation. The given information is true to best of our knowledge.

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CONTENT

Acronym	
ANOVA	Analysis of Variance
AA	Amino Acid
BMI	Body Mass Index
dL	Deciliter
FA	Fatty Acid
FAAS	Flame Atomic Absorption Spectroscopy
FNB	Food and Nutrition Board
ICD	International Classification of Diseases
MDA	Malondialdehyde
mg	Milligram
RDA	Recommended Daily Allowances
SOD	Superoxide Dismutase
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Tetracyclic Antidepressant
TMB	Tetramethylbenzidine
TRP	Tryptophan
UV	Ultraviolet
WHO	World Health Organization
5-HTT	5-Hydroxy Tryptophan
5-HTTLPR	5-Hydroxy Tryptophan Linked Promoter Region
AI	Adequate Intake
CED	Chronic Energy Deficiency
CHD	Coronary Heart Disease
CNS	Central Nervous System
CRH	Corticotrophin Releasing Hormone
CRP	Carbon Reactive Protein
CYP	Cytochrome
DSM	Diagnostic and Statistical Manual
ELISA	Enzyme Linked Immunosorbent Assay
FAAS	Flame Atomic Absorption Spectroscopy
HDL	High Density Lipoprotein
GAD	Generalized Anxiety Disorder
GSIS	Glucose Stimulated Insulin Secretion
HPETE	Hydroperoxyeicosatetraenoic Acid
ICD	International Classification of Diseases
IL-6	Interleukin-6
L	Liter
LDL	Low Density Lipoprotein
min	Minute
NCCLS	National Committee for Clinical Laboratory Standard
NHANES	National Health and Nutrition Examination Survey
NMDA	N-methyl-D-aspartate
RDA	Recommended Daily Allowances
ROS	Reactive Oxygen Species
RP-HPLC	Reverse Phase High Performance Liquid Chromatography
SD	Standard Deviation
SOD	Superoxide Dismutase
TMB	Tetramethylbenzidine
UIL	Upper Intake Level
UV	Ultraviolet
SGOT	Serum glutamic oxaloacetic transaminase
µmol	Micromole

µg	Microgram
BBS	Bangladesh Bureau of Statistics
BDHS	Bangladesh Demographic and Health Survey
BMI	Body mass index
FAO	Food and Agriculture Organization
FCS	Food consumption score
GDP	Gross domestic product
GO	Government organization
PAL	Physical activity level
SGPT	Serum glutamic pyruvate transaminase
ADA	American Diabetes Association
ATP	Adult Treatment Panel
AMORIS	Apolipoprotein-Related Mortality Risk
BAI	Body Adiposity Index
CHD	Coronary Heart Disease
CETP	Cholesteryl Ester Transfer Protein
CD	Cluster of Differenciation
CVD	Cardiovascular Disease
CRP	C- Reactive Protein
DM	Diabetic Mellitus
DCCT	Diabetes Control and Complication Trial
EDIC	Epidemiology of Diabetes Intervention and Complication
FFA	Free Fatty Acid
GAD	Glutamic-acid-decarboxylase
HBAIC	GlycatedHaemoglobin
HSL	Hormone-sensitive Lipase
HDL-C	High Density Lipoprotein Cholesterol
IDF	International Diabetic Federation
IGT	Impaired Glucose Tolerance
IDDM	Insulin Dependent Diabetes Mellitus
LDL-C	Low Density Lipoprotein Cholesterol
LPL	Lipoprotein Lipase
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NHDL-C	Non High density lipoprotein cholesterol
NGSP	National GlycohaemoglobinStandardisationProgramme
NCEP	National Cholesterol Education Program
OGTT	Oral Glucose Tolerance Test
TG	Triglycerides
T2DM	Type 2 Diabetes Mellitus
UKPDS	United Kingdom Prospective Diabetes Study
VAI	Visceral Adiposity Index
VLDL-C	Very Low Density Lipoprotein Cholesterol
VAT	Visceral Adipose Tissue

Abstract

Diabetes mellitus is a global health problem, and it is a major cause of death and disability worldwide. Its global prevalence was about 8% in 2011, predicted to be 10% by 2030. Nearly 80% people living with diabetes in low-middle income countries. In Bangladesh, diabetic prevalence was 9% in 2006-2010, which will be 13% by 2030. Currently, oral hypoglycemic and insulin are used for maintenance of this disorder. Diabetes is a stress-induced chronic disease. Pancreatic beta-cells, which generate insulin, have low antioxidant capacity and are sensitive to oxidative stress. Oxidative stress insults the β-cells to produce insulin, increases insulin resistance and develops glucose intolerance. In type 2 diabetes mellitus, physical activity- improves insulin sensitivity, prevents impaired glucose tolerance, delays onset of diabetes complications, prevent diabetes at high risk group.In addition, diabetes could develop many morbidities and complications such as cardio-vascular diseases (CVD), kidney diseases, retinopathy, neuro-degeration etc. studies reported zinc is insulinomimetic, its supplementation reduces blood glucose level in type II Diabetic patients.

This study is a randomized, double blind placebo-controlled clinical trial, and was conducted among type II diabetic patients attending at Gazipur Diabetic Centre, Gazipur, during June 2015 to December 2018.The research question was- Zinc is insulinomimetic and its supplementation reduces blood glucose level in type II diabetic patients. Therapeutic dose (30 mg) of zinc tablet was supplemented to type II diabetic patients (zinc-group) daily for 3 months and anhydrous lactose tablet (replacement of zinc) was given to the placebo control group (diabetic subjects without zinc) with the same schedule. During the 3 months follow up period, a large number of patients were dropped out and finally 60 and 30 subjects remained in zinc and placebo group respectively. Physical activity with antioxidant rich (fruits and vegetable) and simple carbohydrate restricted diet were advised to the both groups of diabetic patients and had been monitored for 3 months. Socioeconomic and patient's characteristics were recorded in a structured pretested questionnaire in baseline and nutritional status (both dietary and anthropometric) and physical activity level (PAL) for both zinc-group and placebo were recorded in baseline and after 3 months follow up (end line). During the 3 months follow up period, 10 ml blood samples were collected in baseline and following every month to assess biochemical parameters e.g.

fasting plasma glucose (FBG), creatinine, micro albumin, Serum glucose phosphatase transaminase (SGPT), Serum glucose oxalate transaminase (SGOT) and lipid profile (LDL, HDL, TG etc.). However, glycated hemoglobin (A1C), plasma zinc, mealondialdehyde (MDA) and insulin levels were measured in baseline and end line. Standard biochemical methods and statistical software package was employed to analyze the blood samples and data respectively. Descriptive statistics, Repeated Measure ANOVA and Multiple logistic regression models were employed to assess the differences between zinc group and placebo group and to find out the association between different independent and outcome variables, especially, association of plasma zinc with MDA, Insulin A1C and FBG. Level of significance was set at <0.05.

The zinc and placebo subjects were characteristically well matched (P>0.05) with mean age (48.73 vs. 50.27 years), monthly income (BDT 6220.85 vs. 6099.6), and BMI (27.20 vs. 26.9) respectively. Zinc supplementation gradually reduced the fasting blood glucose level from 14.2 to 9.4 mmol/L (P<0.05), while, blood glucose in placebo group was also reduced from 16.0 to 13.3 mmol/L (P>0.05). Plasma A1C value also showed a significant (P<0.05) reduction of blood glucose level (13.4 to 10.9 mmol/L) in zinc group, but not in the placebo group (14.0 to 14.7 mmol/L).Diet restriction of zinc group reduced the calorie intake from 3124.7±453.43 to 2455.0 ±145.36 calorie. Moreover, physical activity increased calorie expenditure from 2180.4±112.4 to 3107.6± 458.0 calorie in zinc group. Furthermore, biochemical and sociodemographic profile of zinc group did not show any association with FBG or A1C level.

The diabetic patients were zinc and insulin deficient and zinc supplementation significantly increased (P<0.05) both the plasma zinc and insulin level from 7.87±4.44 to 76.25±49.34 µmol/L and from 12.03±2.59 to 19.56±4.71 µIU/ml respectively. However, plasma zinc value of placebo group also changed from 7.93±5.21 to 9.83±6.04 µmol/L (P>0.05), which might be due to the advice-only diet restriction and physical activity. This study assured that zinc supplementation increases plasma insulin level which indicates improvement of insulin synthesis and secretion that eventually reduced fasting plasma glucose and A1C level. Contrarily, the change of insulin level (12.06±1.40 to 13.41±2.50 µIU/ml) in placebo group was insignificant. In addition, zinc also significantly

reduced the plasma MDA level from 3.30±0.87 to 2.09±1.03 µmol/L ($P<0.05$), is also a remarkable indication of 'stress alleviation' capacity of zinc. Logistic regression analysis showed that having 18.4 and 17.5 times higher level of serum zinc (>17 µmol/L) and insulin (>17 µIU/ml) respectively in zinc intervention group than placebo at the end line (compared to baseline) could explain positive association of zinc intervention with higher odds of increasing insulin level. Contrarily, zinc group possessed 6.5-times lower serum MDA (≤3 mmol /mL) and 32.5-times lower blood sugar (≤10.5 mmol /L) respectively as compared to the placebo group which indicates lower stressors level in zinc group, associated with higher serum insulin and lower FBG.

Zinc supplementation lowers the blood glucose level. Diet control and physical activity also possibly contributed for the management of diabetes mellitus by controlling insulin secretion and sensitivity. Zinc supplementation improves insulin secretion, influences to reduce oxidative stress, thus, reducing plasma glucose level. It is, therefore, suggested to take therapeutic zinc with hypoglycemic agent for the maintenance of glucose homeostasis in type II diabetic patients.

Chapter one
Introduction

1. Introduction

1.1. Diabetes mellitus

Diabetes mellitus is a metabolic disorder, where body cannot use glucose. Because of insulin deficiency, glucose cannot enter into the cell to be used for metabolism to produce ATP (adenosine triphosphate). The excess glucose in the blood stream is termed as hyperglycemia, it is diabetes. Diabetes mellitus describes a heterogeneous metabolic disorder of multiple etiology characterized by chronic hyperglycemia with distortions in carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both.

Diabetes is a chronic metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The most common is type 2 diabetes, usually in adults, which occurs when the body becomes resistant to insulin or doesn't make enough insulin. In the past three decades, the prevalence of type 2 diabetes has increasedradically in low income countries of all income levels. For people living with diabetes, access to treatment including insulin, is critical to their survival. World Health Organization (WHO) aims to stop this increase of type 2 diabetes by 2025(WHO, 2016).

Diabetes mellitus (DM) is a major public health problem worldwide associated with great deal of morbidity and economic cost. Itis a leading cause of death and disability worldwide (Lozano et al, 2012; Murray et al, 2012). The global prevalence of diabetes was about 8% in 2011, predicted to be increased to 10% by 2030. It is expected that diabetic mellitus will affect 300 million worldwide persons by the year 2030 (Wahhabi et al, 2010). It was estimated that the number of adults with diabetes in the world had increased from 108 million in 1980 to 422 million in 2014 (28.5% due to the rise in prevalence, 39.7% due to population growth and ageing, and 31.8% due to interaction of these two factors. IDF estimates another352.1 million (95% CI 233.5 -577.3 million) people worldwide have a pre-stage of diabetes, called Impaired Glucose Tolerance (IGT), which is anticipated to rise to 531.6 million (95% CI 353.8-883.9 million) in 2045 (IDF, 2019).On global scale, diabetes hits particularly the "middle-aged" people of age between 40 to 59 years, which makes stern economic and social implications (IDF 2017; 2019). Nearly 80% of people with diabetes live in low- and middle-income countries. In 2011, China was home to the largest number of adults with diabetes (90.0 million, or 9% of the population), followed by India (61.3 million, or 8% of the population) and in

Bangladesh; it is 8.4 million, or 10% of the population (IDF, 2013). In Bangladesh, prevalence of diabetes among adults increasing largely, from 5% in 2001 to 2005 to 9% in 2006 to 2010, and it will be 13% by 2030(Saquib et al, 2012).An estimated 10 million people in Bangladesh have diabetes; one in ten adults. WHO stated 83 % population of age group 25-65 never checks for? For an effective control and prevention of diabetes; 87% of Bangladeshisis in status of compliance, but not improved in the last 14 years. Around 33% people age over 35 are diabetic or pre-diabetic, only 12% of them are under control. Approximately 17% of men and 23% of women were identified to have impaired fasting glucose or impaired glucose tolerance, collectively in intermediate hyperglycemia .Only 25% of diabetics are aware of their status, women with diabetes were 37% less likely than men to know that they were diabetic and, even among known diabetics, 75% had suboptimal control of the condition According WHO (2017) data diabetes Mellitus Deaths in Bangladesh reached 40,142 or 5.09% of total deaths.

Chronic hyperglycemia is believed to play a pivotal role in the development of diabetic complications. It was found that hyperglycemia incites a number of mechanisms that induce overproduction of reactive oxygen species (ROS). DM is associated with an increased level of free radicals, disturbances of the enzymatic antioxidant defense system. Consequently, these abnormalities lead to a redox imbalance called oxidative stress (Preedy et al, 2011).

1.2. Oxidative stress and diabetes

Diabetes is stress induced metabolic disease. Oxidative stress is now addressed as the key etiology of several chronic diseases including cardiovascular system (CVS), diabetes, neurogeneration, kidney complication, ophthalmic disorder, and even cancers (Thomas, Philipson, 2015).

- Oxidative stress is involved in type II diabetes & its complications (Thomas, Philipson, 2015).
- The oxidative stress reduces insulin secretion, increases insulin resistance and develops glucose intolerance. Diabetes itself generates excess reactive oxygen species (Lloyd et al, 2005; Midget et al, 2010).
- Pancreatic beta-cells have low antioxidant capacity and are sensitive to oxidative stress (Lloyd et al, 2005).
- Oxidative insult of pancreatic beta-cells has now focused as the key etiology of diabetes and insulin resistance (Lloyd et al, 2005).

- Obesity, unhealthy dietary habits, sedentary life style and genetic factors are considered as important risk factors in the development of T2DM.
- Zinc, as antioxidant, fight against stress and thus reduces oxidative stress.

1.3. Classification/type of diabetes

There are mainly two types of diabetes mellitus (type 1 and type 2 diabetes mellitus), though there are other rare forms of diabetes mellitus. Type 1 diabetes (insulin-dependent diabetes) is characterized by insulin deficiency resulting from pancreatic beta cell destruction, etiology of which is either immune mediated, related to either physical destruction of the diabetes is type 2 diabetes, which accounts for over 90% of all diabetes cases, presents a spectrum of metabolic abnormalities with prominent insulin resistance and relative insulin deficiency. However, on the basis of insulin availability, diabetes are categorized into

i. Type 1 diabetes (T1D)- beta-cell destruction, leading to absolute insulin deficiency
ii. Type 2 diabetes (T2D) - insulin resistance with relative insulin deficiency, predominantly insulin secretory defect in the beta cells of pancreas.
iii. Gestational Diabetes mellitus (GDM) - glucose intolerance, first recognized during pregnancy.
iv. Other specific types- Genetic defects of beta -cell function, insulin action. Diseases of the exocrine pancreas, Endocrine pathies, Drug- or chemical-induced)

Whatever is the case, the end result is diabetes (Thomas and Phloipson, 2015).

Type 1 diabetes

It isalso previously known as insulin-dependent, juvenile or childhood diabetes, and is characterized by deficient insulin production, so requires daily use of insulin. Its cause is not known and is not preventable with current knowledge. Its symptoms include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes, and fatigue, which may occur suddenly.

Type 2 diabetes

It isformerly called non-insulin-dependent, or adult-onset resulting from ineffective use of insulin. Itis prevalent in the majority of people around the world, and is largely because of obesity, overweight, and physical inactivity or sedentary lifestyle. Its symptoms may be similar to those of type 1 diabetes, but less marked. As a

result, this disease may be diagnosed after several years of onset, until complications developed. It is usually occur in adults, but currently also occurring increasingly and frequently in children.

Gestational diabetes

Gestational diabetes is hyperglycemia with high blood glucose value, but below those of diagnostic diabetes. It occurs during pregnancy. Women with gestational diabetes are at an increased risk of complications during pregnancy and at delivery. The mother and their children are at increased risk of type 2 diabetes in the future. Gestational diabetes is diagnosed through prenatal screening, rather than through reported symptoms.

Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG).

The (IGT) and (IFG) are intermediate conditions of diabetes. People with IGT or IFG are at high risk of progressing to type 2 diabetes, which isnot inevitable.

Other types of Diabetes Mellitus

Tissue receptors tyrosine does not responding to insulin, even having insulin levelnormal. Genetic mutations autosomal or mitochondrial can lead to defects in ß-cells function (Wellen et al, 2003). Any disease that causes much damage to the pancreas may create diabetes in the person, for example- chronic pancreatitis and cystic fibrosis. Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes, which can be treated with reducing excess hormone. Many drugs impair insulin secretion and some toxins damage pancreatic β-cells.

1.4. Pathogenesis of Diabetes

Multipleetiologies are involved in the pathogenesisof the development of diabetes, which include

(i) Annihilationof pancreatic β- cells resulting in insulin deficiency,

(ii) Developing resistance to insulin action, and

(iii)Abnormalitiesin metabolism of carbohydrate, fat and protein owing to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin

These etiological defects, disorders or processes induce diabetes mellitus.

Geneticfactors contribute to the etiology of type 2 diabetes or diabetes mellitus Radha et al, 2007). The concordance of type 2 diabetes in monozygotic twins is about 70% compared to 20–30% in dizygotic twins. The lifetime risk of developing it is 40% in the offspring of one parent with the disease, but the risk approaches 70% if both the parents are affected. Women with gestational diabetes are at an increased risk of developingtype 2 diabetes mellitus after pregnancy, while their offspring are prone to develop childhood obesity, with type 2 diabetes later in life. Fetal exposure to maternal diabetes is associated with a higher risk of abnormal glucose homeostasis in the offspring and among patients with gestationaldiabetes, a higher frequency of diabetes history has been reported in the mothers than in the fathers (Fetitaet al, 2006).

Diabetes can be treated and its consequences can be avoided or delayed with diet, physical activity, medication and regular screening and treatment for complications (WHO, 2018).According to the IDF statistics, presently every seven seconds someone is estimated to die from diabetes or its complications.

Lifestyle changes due to urbanization including diet, physical inactivity, stress, smoking and alcohol consumption also stem type 2 diabetes. The transition to modern diet lead to increased insulin resistance alteringthe glucose levels in the body along with decreasedphysical activity and sedentary life styles leading toincreased prevalence of the type 2 diabetes (Kowall et al, 2010). Reduced fiber intakeand increased consumption of animal fats and processedcarbohydrates can augment type 2 diabetes, and traditional diet of cereal-based, rich in fiber and low in saturated fat, cholesterol and meat can relief type 2 diabetes.

1.5. Diagnosis of Diabetes mellitus

The World Health Organization () and the Expert Committees of the American Diabetes Association (ADA) outlined the criteria for the diagnosis of hyperglycemia for diabetes mellitus as

Parameter	**2-hour glucose**	**Fasting glucose**	**HbA1c**	
Unit	mmol/l(mg/dl)	mmol/l(mg/dl)	mmol/mol	DCCT %
Normal	<7.8 (<140)	<6.1 (<110)	<42	<6.0
Impaired fasting glycaemia	<7.8 (<140)	≥6.1(≥110) &<7.0(<126)	42-46	6.0–6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	42-46	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)	≥48	≥6.5

Whatever is the case, the end result is diabetes.

1.6.Treatment and management of diabetes mellitus

Diabetes mellitus isa chronic lifetime metabolic disorder. Aim of diabetes treatment is to maintain normal glycaemia, also to reduce micro- and macro vascular complications, and monitoring complications. Therapeutic approach includes non-pharmacological strategy and pharmacological approach. The first one addresses to medical nutrition therapy, body weight maintenance, and balance diets containing low glycemic low carbohydrates and low saturated fats and calorie restriction form weight loss. The pharmacological therapy includes oral hypoglycemic agents and injectable insulin.

1.6.1. Oral hypoglycemics

The oral hypoglycemic agents currently using are

- Sulfonylureas- Glibenclamide, Glipizide
- Biguanides- metformin
- Meglitinide analogs- Repaglinide, Nateglinide
- Thaizolidinediones- Pioglitazone
- Alphaglucosidase inhibitors - Acarbose
- Dipeptidyl Peptidase-IV Inhibitors- Sitagliptin, Vildagliptin.

1.6.2. Insulin therapy

Types of insulin used in the management of glycemic index are

- Rapid acting (15-30 min)- Aspart, lispro
- Intermediate acting (2-4 hrs)- Lente
- Long acting (4-10 hrs)-Glargine
- Short-acting insulins (30–60 minutes)-Humulin R (regular), Novolin R (regular).

Type 2 diabetes is characterized by insufficient secretion of insulin from the β-cells of the pancreatic islets. Insulin resistance is increased during obesity. Treatment of type 2 diabetes starts initially of dietary control and lifestyle modifications, followed by oral hypoglycemic agents. Oral hypoglycemic agents are given to stimulate pancreatic β-cells to synthesis and secrete insulin or to reduce need of insulin reducing glycemic load. When β-cells are damaged or could not produce insulin, then insulin therapy is required.

1.7. Diet control and exercise

The Diet control and exercise are essential for effectiveness of hypoglycemic agents.

1.7.1. Diet control

Diet control is required for maintenance of glycemic index in diabetes mellitus (DM) are

(i) appropriate daily calorie intake,
(ii) nutritional balance diet and
(iii) Appropriately divided meals taken at designated time interval (Hiroshi, 2001).

The diabetic diet should contain –

(i) carbohydrate restricted low GI, high fiber complex diet to reduce and slow insulin need and
(ii) Antioxidant rich vegetables and fruits to protect against oxidative stress.

1.7.2. Exercise or Physical activity

Like diet control, physical activity or exercise is another key component for control or maintenance of diabetic control (Hiroshi, 2001).The physical activity

(i) makes movement of skeletal muscles resulting energy expenditure,
(ii) Activate/tune human organs,
(iii) help organs, muscles, bones, arteries more efficient, and thus,
(iv) Reduce chances of getting illness or disease.

In type 2 diabetes mellitus, physical activity

(i) improves insulin sensitivity (WHO/IDF, 2003)
(ii) prevents impaired glucose tolerance (Robertson et al, 2020),
(iii) delays onset of diabetes complications,
(iv) Prevent diabetes at high risk group (Zinman et al, 2003).

1.8.Insulin synthesis and secretion

Insulin is made of 2 peptide chains - A chain and B chain, which links together by two disulfide bonds, and an additional disulfide is formed within the A chain. The A chain consists of 21 amino acids and the B chain of 30 amino acids. Insulin is synthesized in the Beta cells of the islets of Langerhans. Insulin mRNA is translated into preproinsulin, which generates proinsulin. End peptidases cut out a connecting peptide (c-peptide) between the A and B chains to make the mature form of insulin, which is secreted by exocytosis from the β-cells by diffusion.

Insulin secretes in in two phases- pulsatile release (rapid onset) clearing absorbed nutrients from the blood following a meal, and protracted release (longer): Long term insulin release for glucose uptake such as for cell growth, cell division, stimulating protein synthesis and DNA replication. Insulin binds to a highly specific insulin receptor on cell surfaces.

Insulin is synthesis in β-cells as preproinsulin to proinsulin, which in turn is converted to insulin , and stored in secretary granules, where waiting to release on demand. Insulin synthesis is regulated at transcriptional and translational level. Insulin secretion takesplace is a chain of steps in β-cells that make to blend of secretory granules with the plasma membrane in response to glucose. Fatty acids and amino acids augment glucose-induced insulin secretion. In addition, some hormones, such as melatonin, estrogen, leptin, growth hormone, and glucagon like peptide-1 also regulate insulin secretion. Thus, the β-cell is a metabolic center in the body, connecting nutrient metabolism and the endocrine system. CAMP gives signal to regulate the insulin secretion (Fu et al, 2013).

1.9.Importance of zinc in type 2 diabetic patients

Zinc is one of the essential trace elements that is required to maintain the normal physiological function of all forms of life (Saradesai et al, 1998). Zinc occurs within a great variety of foods of both animal and plant origin. Zinc within animal products is more readily available than that within plant products (Zheng et al, 1993). Zinc has close interrelationships with the endocrine system and it is essential for normal growth, reproductive function, immune function, and glucose metabolism. Zinc deficiency has now been recognized to be associated with many chronic illnesses (Prasad et al, 2003). Diabetes mellitus (DM) is one of the diseases, which affect zinc homeostasis in different ways. The relationship between diabetes, insulin, and zinc is complex with no clear cause and effect relationship. The predominant effect

of diabetes on zinc homeostasis is hypozincemia, which may be the result of hyperZincuria or decreased intestinal absorption of zinc or both (Chausmer et al, 1998).

Zinc has an important role in the glucose utilization by muscle and fat cells (Song et al, 1998). It is required as a co-factor for the function of intracellular enzymes that may be involved in protein, lipid and glucose metabolism (Saguaro et al, 2001). Zinc may be involved in the regulation of insulin receptor-initiated signal transudation mechanism and insulin receptor synthesis (Tang et al, 2001). Zinc also plays a key role in the synthesis, storage, and secretions of insulin by pancreatic tissue, and it accounts for the conformation integrity of insulin in its hexametric crystalline form (Jindal et al, 1992). Zinc may participate as an integral component of several antioxidant enzymes. Many of the complications of diabetes may relate to an increase in intracellular oxidant and free radicals associated with decrease in intracellular zinc and zinc dependent antioxidant enzymes (Szaleczky et al, 1997).

1.10.Zinc, diabetes and antioxidant defense

Zinc provides defense against diabetes. Zinc is insulinomimetic- acts like insulin, decreases blood glucose, HBA1 and cholesterol levels (Khanam et al, 2018), deficiency of zinc reduces insulin secretary reserve and makes glucose intolerance (Kim J Lee et al, 2012), and stimulates glucose oxidation and glycemic control by modulating insulin signaling pathway (Norouzi et al,2018). Zinc plays vital role in the synthesis, storage and secretion of insulin and its conformational integrity, and its deficiency affects the ability of islet cell to produce and secrete insulin (Chausmer, 1998). Zinc syntheses proinsulinin pancreatic β-cell, which then makes a zinc containing insulin hexamer. Zinc ions also enhance proinsulin'ssolubility and render insulin insoluble-microcrystalline character of the precipitated insulin granule (Omar et al, 2001).

Zinc provides defense against oxidative stress. It is important for over 300 enzymatic reactions and is a part of more than 2000 zinc-dependent transcription factors and zinc-metaloenzymes. Zinc-metaloenzymes such as superoxide dismutase, protect cells and tissues from free radicals insults (Kaur et al, 2014).Diabetes disrupts zinc-homeostasis. Zinc has strong antioxidant potential, it acts as a cofactor of the superoxide dismutase enzyme which regulates detoxification of reactive oxygen species, thus protecting against the oxidative stress induced by chronic hyperglycemia (Cruz et al, 2015). Zinc also inhibits α-ketoglutarate-dependent mitochondrial respiration that suggests Zn^{2+} can interfere

with mitochondrial antioxidant production and may also stimulate production of reactive oxygen (Gazaryan et al,2002).Zinc supplementation has been shown to improve type 2 diabetes symptoms (Begin-Heick et al, 1985). Dietary zincsupplementation has been reported to attenuate hyperglycemia and hyper insulinemia in diabetic patients (Simon et al, 2001).The effects on the diabetic phenotype vary but globally, scientists observed beneficial effects especially in decreasing HbA1c levels and cholesterol (El Dib et al, 2015). Zinc supplementation affects the insulin response differentially, depending on the patient genotype for the SLC30A8 gene encoding the zinc transporter ZnT8 (Maruthur et al, 2015).

1.11.Zinc in insulin synthesis in β-cells

Zinc is essential for the effective synthesis of insulin hormone, which is arranged in a regular crystalline structure comprising Zn ions in secretory vesicles. Each insulin molecule is linked with 2–4 Zn atoms. A zinc/insulin complex is formed for slow release of insulin into the bloodstream (Prasad et al, 1998).

The healthy pancreas has high zinc content, which is greatly decreased in diabetic patients. Zinc presents in pancreatic β-cells, and concentrates within the dense core insulin secreting granules (ISG) at around 10 to 20 uM (Hutton et al, 1983). Zn^{2+} ions are an essential for both insulin processing and storage.

1.12. Diabetes, immunity and zinc

Diabetis is a chronic lifetime diseases. Clinical use of zinc benefits health, including may settle diabetes (McClung Cai et al, 2014). It can repair the organs damage induced by oxidative stress. Diabetic is considered as immune- compromised metabolic complication. Zinc plays vital function in immune function. Zinc status is an important determinant of cell-mediated immunity, it may modulate immune system in diabetes (Mooradian et al, 1988; Eliashiv et al, 1978). Zinc therapy has been reported to make it normal, even zinc enhances immunity by increasing percentage of CD4 T–cells. Zinc adds to insulin signaling, glucose use, maintain lipid metabolism and cell functions. Zinc deficiency induces chronic disease including diabetes, which itself also leads zinc deficiency. Zinc therapy prevents start or development of diabetic cardiomyopathy, also averts development and or the progression of diabetic nephropathy.

1.13. Inflammation, zinc and diabetes

Zinc acts as an anti–inflammatory agent (Mooradian et al, 1988: Khan et al, 2013). A large body of investigations has reported function of zinc in the regulation of metabolic syndrome. It controls cytokine expression, suppresses inflammation, also makes activation of antioxidant enzymes to scavenge reactive oxygen species reducing oxidative stress (Olechnowicz et al, 2018). Inflammation associated with insulin resistance and diabetes (Dandona et al, 2004; Grimble et al, 2002). Zinc deficiency occurs in inflammation and type 2 diabetes (Lehmer et al, 2005). Which is due to high IL-6 production that associates with insulin resistance, hyperglycaemia and dyslipidemia. The -209 A/G MT2A polymorphism is involved in chronic inflammation (high plasma IL-6), hyperglycaemia, enhanced HbA1c, which are manifested by marked zinc deficiency. Therefore, zinc therapy may overcome the diabetic inflammation.

1.14.Zinc supplementation to type2 diabetic patients

Zinc concentration in the islet of β-cells is related to synthesis, storage and secretion of insulin, deficiency of which affects the ability of islet cell to produce and secrete insulin (Chausmer et al, 1998). Zinc on cellular homeostasis. It stimulates glucose uptake, induces lipogenesis in adipocytes, makes tyrosine phosphorylation of insulin / IGF-1 receptor and insulin receptor substrate-1, activates epidermal growth factor receptor, Inhibits PTP (protein tyrosine phosphatases), activate mitogen activated kinases (MAPKs), C- nonterminal kinases, and increase in glycogen synthesis.

Zinc supplementation has been found to reduce the progression and complications of diabetes by reducing oxidative stress and apoptosis (Daga et al, 2009; Ota et al, 2004).In the context of dietary zinc supplementation to ameliorate diabetes complications, there have been some contradictory results on zinc's efficacy in glucose hemostasis (Jansen et al, 2009; Hwang et al, 2011; Miao et al, 2013; Zhao et al, 2012).

1.15. Hypothesis

Zinc is insulinomimetic, acts like insulin. Zinc supplementation reduces blood glucose level indiabetic mellitus, type 2 Diabetes.

1.16. Rationale of the study

Zinc provides defense against diabetes. Zinc is insulinomimetic; acts like insulin. It decreases blood glucose.Zinc deficiency reduces insulin secretary reserve and makes glucose intolerance. Zinc stimulates glucose oxidation and glycemic control by modulating insulin signaling pathway. Zinc plays vital role in the synthesis, storage and secretion of insulin and its conformational integrity. Zinc deficiency affects the ability of islet of β-cell to produce and secrete insulin. The pan creative, β-cell is highly sensitive to oxidative stress.Zinc is required for insulin synthesis in β-cell. Zinc ions also enhance proinsulin's solubility and render insulin insoluble-microcrystalline character of the precipitated insulin granule.In light of insulinomimeticand stress alleviating potential of therapeutic zinc, this study aimed therapeutic supplementation of zinc in type 2 diabetes to reduce blood glucose with placebo supplementation.

1.17. Objective of the study

This study aimed to investigate the effect of therapeutic zinc on blood level in type 2 diabetics. To this end, the specific objectives have designed to

(i) assessment of the sociodemographic characteristics of the study population
(ii) assessment of nutritional status with anthropometrics
(iii) supplement ation of zinc (therapeutic dose) to type 2 diabetes
(iv) advice, monitor and controll the diabetes diet and physical activity
(v) analyze biochemical profiles-creatinine, micro albumin and SGPT, SGOT and lipid profile; and
(vi) analyze plasma zinc, insulin and stress biomarkers at baseline and follow up times with standards methods and finally
(vii) analyze the influence/effect/association of
    a) (i) zinc on insulin level, (ii) insulin on plasma glucose level, and (iii) Zinc on malondialdehyde
    b) diet control/restriction on plasma glucose level, and
    c) physical activity level on plasma glucose*content*

Chapter two

Materials and Methods

2. Materials and Methods

2.1. Study design

The study was designed as a placebo-controlled clinical trial conducted on type 2 diabetic patients. Zinc tablet (30mg; Square Zinc®, Zinc Sulfate Monohydrate USP equivalent to 20 mg Zinc) was supplemented to the type 2 diabetics every day for 3 months and placebo to the control group with follow up for one month, two month and three months (Hyun-Meet et al, 2009; Roussel et al, 2003; Morsi et al, 2012).The research work was categorized into

Part I: Socio-demographic, anthropometric (BMI), dietary habit (24 hour recall) and physical activity (24 hour physical activity) data were collected and recorded,

Part II: Plasma FBS, HbA1c, creatinine, micro albumin, SGPT, SGOT, and zinc and insulin and MDA levels were analyzed at baseline, follow up times, and

Part III: influence/effect/association of

a. (i) zinc on insulin level,
b. (ii) insulin on plasma glucose level, and
   (iii) Zinc on malondialdehyde,
b) diet control/restriction on plasma glucose level, and
c) Physical activity level on plasma glucose content.

2.2. Study population

This study was conducted among type 2 diabetic (T2D) patients attending Gazipur Diabetic Centre, Gazipur, during the period of June 2015 to December 2018. Informed consent was taken from each of the subjectsat the beginning of the study.

Ethical approval was obtained from ethical committee of the Faculty of Biological Science, University of Dhaka.

2.3. Questionnaire development

A questionnaire was designed, prepared and pretested to collect and record the socio-demographic, anthropometric, dietary and physical activity data, and to record the biochemical analytical results (Appendix A).

Picture 1: Gazipur Diabetic Centre, Gazipur

2.4. Sample size estimation

Sample size for RCT (comparing two means; Morsi et al, 2012)

$$n = (Z_{\alpha/2} + Z_{\beta})^2 \times 2(\sigma)^2 / (\mu^1 - \mu^2)^2$$
$$n = (Z_{\alpha/2} + Z_{\beta})^2 \times 2(\sigma)^2 / (\mu^1 - \mu^2)^2$$

$$= (1.96 + 0.84)^2 \times 2(0.99)^2 / (0.61 - 0.10)^2$$
$$= (2.80)^2 \times 2(0.99)^2 / (0.51)^2$$
$$= 7.84 \times 1.96 / 0.26$$
$$= 59.10 \sim 59$$

Where
n = sample size required in each group,
μ1 = mean change in glycemic control in treatment group = 0.71 (Morsi et al 2012)
μ2 = mean change in glycemic control in placebo group = 0.1
μ 1-μ2 = clinically significant difference = 0. 61
ó = standard deviation = 0.99
$Z_{\alpha/2}$ =level of significance, for 5% this is 1.96
Z_{β} =power, for 80% this is 0.84

Based on this formula, the sample size required per group is 59. Hence total sample size required is 118. Considering a dropout rate of 25%, total sample size required is eightynine.

A sample size of 89 subjects is sufficient to detect a clinically important difference of 0.61 between groups in improvement of glycemic control assuming a standard deviation of 0.99 and using a two tailed t-test of difference between means with 80% power and at 5% level of significance.

2.5. Recruitment of study population

Type 2 diabetic patients of aged 35 year to ≥ 51 year were recruited under defined criteria. The patients and the guardians were briefed about the objectives of the study and written consent was taken from each of the participants or the attendant (if required).

Inclusion criteria: Diabetic patients, who were

- in insulin treatment but did not need to change the treatment regimen,
- voluntarily willing to participate in this clinical study, and
- Agreed to comply the treatment given.

Exclusion principle: Diabetic patients, who were

- with some co-morbid illness, such as uncontrolled hypertension, heart disease and any other serious debilitating illness, and
- Uncertain to continue the treatment adherence.

2.6. Grouping of recruited type 2 diabetics

The recruited diabetic patients were divided into zinc: placebo nearly 2:1ratio.

I. Zinc group, who received zinc tablet (30mg) once every day for 3 months with constant follow up over phone and diabetic center visit, and

II. Placebo group- diabetic patients, who received placebo with the same schedule of zinc group.

The experiment was conducted as designed for follow up and investigation.

2.7. End stand of zinc and placebo group

During the study period, a large number of patients was dropped out at every the follow up and finally at end line. In course of follow up time and investigation, a large number of patients were dropped out, and ultimately. It stands as

- Zinc group type 2 diabetics 60 (sixty), and
- Placebo group type 2 diabetics 30 (thirty).

In Random Clinical Trial (RCT) and Clinical Trial (CT) studies, this type of dropping the experimental human subjects is very common and the number of study case and placebo groups are also prevalently frequent (Oh, Yoon etal, 2008. case: control-44:34; Roussel et al, 2003. case: control-27:29; Morsi et al, 2012. case: control 30:30; Islam et al, 2004. 27-33, 253:100).

2.8. Investigation schedule

An investigation schedule was made to conduct the research as designed.

Information	Baseline visit	1st FollowUp	2nd FollowUp	3rd Follow Up
Informed consent form	✓			
Collection of demographics	✓			
Medical history taking	✓			
Physical examination	✓			✓
FBS	✓	✓	✓	✓
ABS	✓	✓	✓	✓
HbA1c	✓			✓
Lipid profile	✓			✓
SGPT/SGOT	✓			✓
plasma creatinine	✓			✓
Urinary micro albumin	✓			✓
plasma insulin	✓			✓
plasma zinc	✓			✓
MDA test	✓			✓
Dietary habit	✓			✓
Physical activity	✓			✓

2.9. Life style of diabetic patients

In order to assess the life style of the diabetic patients, detailed information on sociodemography, anthropometry, diabetic history, treatment taking, dietary habit, and physical activity were collected with an interviewer administered questionnaire. The information thus collected were compiled and analyzed statistically using SPSS Software program (Version-21).

2.10. Collection of research data and blood specimen

The required information and blood specimen were collected from every type 2 diabetic participant by the researcher and enumerators and recorded in the questionnaire, which were then made analysis.

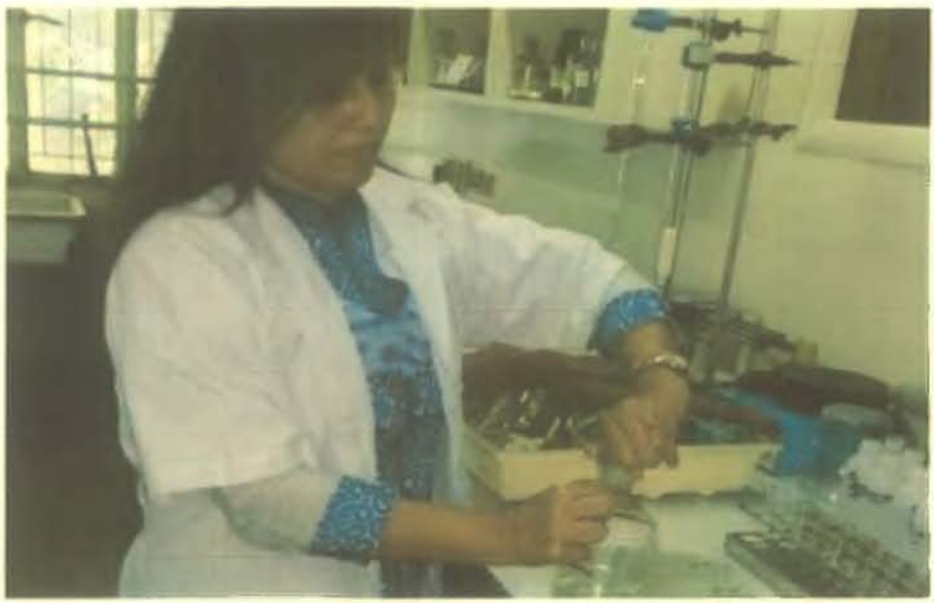
2.10.1. Information collection and data processing

Sociodemographic, anthropometric, dietary and physical activity data were collected and recorded in the pretested questionnaire. Researcher and twoenumerators collected the data by direct interviewing the subjects, and the attendant (when required).

2.10.2. Collection of blood specimen

A 10 ml venous blood sample was collected aseptically from the antecubital vein of each of the participating diabetes in a heparin tube by a paramedic of the diabetic centre, and it was put in cool box. Immediately, blood sample was processed to separate plasma, which was then aliquoted into eppendorfs and stored at -40°C for analysis of biochemicals- FBS, HbA1c, plasma creatinine, micro albumin, SGPT, SGOT, cholesterol, triglyceride, HDL, LDL, VLDL, MDA, Insulin, and zinc.

Insulin analysis was carried out within 7 days of blood collection.



Picture 2: Researcher working in the analytical lab, INFS

2.11. Data analysis

Socio-demographic, anthropometric (BMI), dietary habit (24 hour recall method) and physical activity (24 hour activity), and their effect on blood glucose level and HbA1c were analyzed and interpreted with the use of statistics. The data collected were processed by removing illegitimate codes, reducing logical inconsistencies, dropping improbabilities and by solving ambiguities. The processed data were tabulated on the basis of similarities and intervals by using IBM SPSS Statistics 21 software packages and Microsoft excel were used for data entry. Descriptive statistics (frequencies, cross tables, descriptive) and compare means (t-test) were used to calculate all variables. Values were expressed as frequency, percentage, mean and standard deviation. Chi-square and fisher's exact test were used to assess association or influence. The significance of difference was tested using one sample t-test with the 5% level of confident interval, test statistic and its variance for categorical variables. Mauchly's test was applied to estimate the level of significance when a cell value of any category was less than five.

Nutritional status was assessed using Body Mass Index (BMI: weight in kilogram/height in meter^2) in accordance with reference of (Keizer et al, 2011).

2.12. Collection of anthropometric, dietary and PAL data

The researcher and enumerators collected the Socio-demographic, anthropometric, dietary habit and physical activity data by direct interview of the participants and attendant (if required).

2.12.1. Measurement of anthropometric indices

Body weight and height of the zinc and placebo group diabetic patients were measured while the subjects were not wearing shoes. A researcher adult metric scale (Detector Scale Inc. Brooklyn New York USA) was used to measure the height and weight of each study subjects. Body mass index (BMI) and chronic energy deficiency (CED) were categorized using standard method (Golden et al, 2000; WHO, 2020).

$$BMI\ (kg/m^2) = Weight\ in\ kg/Height\ in\ (meter)^2$$

Classification of chronic energy deficiency (CED) (WHO 2020)

BMI	≤16.0	16.0-16.9	17.0-18.4	18.4-25.0	≥25.0
CED	III	II	I	Normal	Obese

2.12.2. Dietary habit and physical activity

Dietary habit by taking 24 food intake was collected. Food class, food source and nutrient content were collecte in the questionnaire (Keyzer et al, 2011). Physical activity was obtainedl by recording 24 hour activitylevel morning to morning (Welk et al, 2014).

2.12.2.1. Dietary habit

Dietary intake data were collected by recording the food intake during the 24 hour (Keyzer et al, 2011). Repeated 24-hour recalls versus dietary records for estimating nutrient intakes in a national food consumption survey.

2.12.2.2.Physical activity level

It was obtained by monitoring the different activities done during the last 24 hours, morning to morning (Gregory et al, 2015).

2.13. Biochemical analysis at baseline, follow ups

The plasma fating glucose, HbA1C, lipid profiles, liver function and kidney function and plasma zinc, insulin and malondialdehyde were analyzed by standard methods using kits and reagents, which are outline as follow.

Analytical method employed

Bio chemicals	Method	Reagent (kit)	Procedure	Reference
Blood glucose	Glucose oxidase (GOD-PAP)	Human, Germany	5µl plasma+250µl reagent, 10min at 37°C, read absorbance at 500nm &Cal concentration.	Barham, Trinder, 1972
HbA1c	Nyco Card Test method	Randox biochemical, UK	55µl whole blood + Reagent R1 + 3min at 25°C + 25 µL R2, incubate at 10sec, read in Nyco Card Reader	Lenzi et al, 1987
Creatinine	Colorimetric method	Human,Germany	100µl plasma+1000 µl reagent incubate for 30sec, read absorbance at 510nm	Bartels et al, 1971
Micro-albumin	Colorimetric BCG-method	Human,Germany	10µl+1000 µl reagent 5 min at 25°C read absorbance at 546nm & Cal albumin concentration.	Rod key et al,1964
SGPT	Liquiuv method	Human,Germany	200µl plasma+1000µl reagent +incubated at 37°C, read absorbance at 340 nm	Clin.Chim. et al, 1980
SGOT	Liquiuv method	Human,Germany	200µl plasma+1000µl reagent +incubated at 37°C, read absorbance at 340 nm	Clin.chim.e t al, 1976
Cholesterol	Enzymatic method CHOD-PAP	Randox biochemical, UK	5µl plasma+ddH_2O+standard 5µl+500µl reagent R1, incubate at 37°C for 60 min, read absorbance at 500nm &calconcentrtion.	Tietz et al, 1990
Triglyceride	Enzymatic colorimetric GPO-PAP	Randox biochemical, UK	5µl plasma+ddH_2O+standard 5µl+500µl reagent R1, incubate at 37°C for 60 min, read absorbance at 500nm &cal concentration.	Tietz et al, 1990
HDL, LDL,VLDL	Enzymatic colorimetric GPO-PAP	Randox biochemical, UK	5µl plasma+ddH_2O+standard 5µl+500µl reagent R1, incubate at 37°C for 60 min, read absorbance at 500nm &cal concentration.	Tietz et al, 1990
MDA	Spectrophotometric	Reagents	1ml plasma + saline0.6ml+2ml reagent+ boiling for15min at100°C, centrifuge at 3000rpm for 10min, read absorbance at 550 nm &cal concentration	Buege, Aust. 1978
Insulin	Enzyme- linked sandwich method	DRG, Germany	25µl std+25µl enzyme conjugate, incubate 30min +50µl enzyme complex+50µl enzyme substrate, read absorbance 450±10 nm plate reader, calconc..	Flier et al, 1979
zinc	Atomic absorption spectrometric	Shimmadzu	plasma diluted x10 , extinguished in flame AAS, read absorbance at 213.9nm, calconc	Hossain et al, 2007

2.13.1. Estimation of fasting blood glucose level

The Glucose oxidase GOD-PAP method (Barham, Trinder, 1972) was used to estimate blood glucose level using a kit (Human, Germany).

Glucose oxidase (GOD) enzyme reacts with glucose and produces gluconic acid and hydrogen peroxide (H_2O_2), which in presence of 4-amino anti purine and phenol produces a colored sodium azide.Color intensity is proportional to the concentration of glucose content. This reaction is catalyzed by peroxidase (POD).

The kit contained

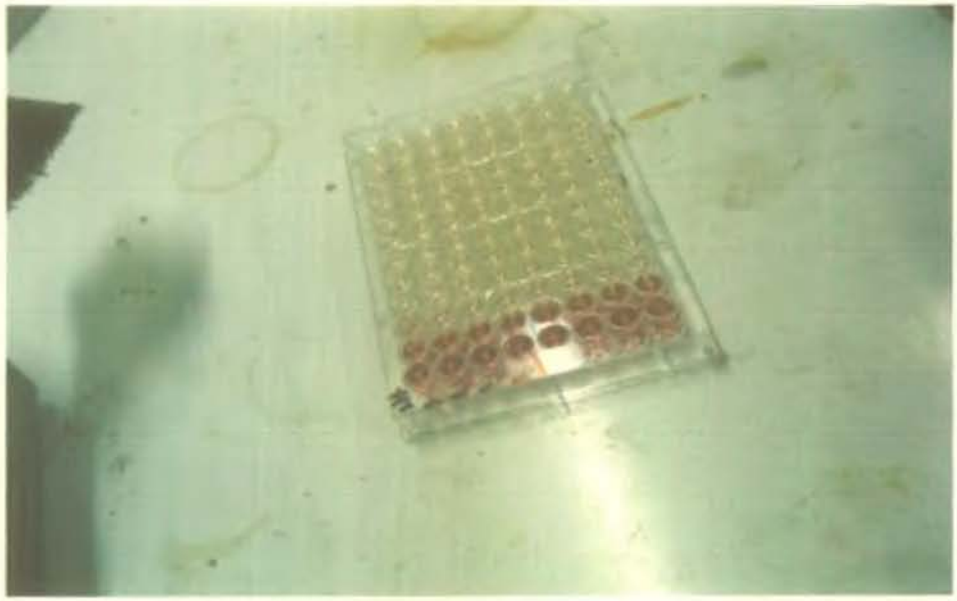
Phosphate buffer, pH7.5	100 mmol /l
4-Amino anti pyrine	0.25 mmol/l
Phenol	0.75 mmol/l
Peroxidase	≥1.5KU/l
Glucose oxidase	≥1.5KU/l
Mutarotase	≥1.5KU/l
Sodium azide	0.095

A standard curve was prepared using absorbance or OD versus different known concentrations (2, 4, 6, 8, 12, 16, 20) of glucose.

Five µl plasma was taken in the micro well of microtitre plate and 250 µl of working reagent was added and incubated for 10 minutes at 37^{0}C, absorbance was read at 500nm.

Calculation $(A_{sample} / A_{standard}) \times$ Concentration of the standard (5.5mmol/l)
=Glucose Concentration of the sample

Picture 3: Estimating plasma glucose



Picture 4: Analysis of plasma glucose

2.13.2. Glycated Hemoglobin HbA1c

Plasma HbA1c was estimated by Nyco Card Test method (Lenzi et al, 1987). It is aboronate affinity assay. The kit reagent makes lysis of erythrocytes to precipitate hemoglobin, which conjugates with blue boronic acid, which then binds cis-diols of glycated hemoglobin. The excess colour was washed out. The precipitated is evaluated by measuring the blue (glycated hemoglobin) and the red (total hemoglobin) colour intensity with the Nyco Card Reader III, the ratio between them being proportional to the percentage of HbA1c in the sample.

The kit contained

24 test kit TD/Test Device.
Plastic device containing a membrane filter
R1/ Reagent
Glycinamide buffer containing dye-bound boronic acid and detergents
R2/washing solution
MorpholinebufferdNaCl solution and detergents
Capillary tube or pipette (5µL) for sample collection
Capillary tube holder
Pipette (25µL) and Pipette tips
Blood samples with anticoagulant (EDTA, heparin and NaF) can be stored up to 10 days at 2-8^{0}C before analysis
Nyco Card READERII

Five µL whole blood was added to R1/Reagent in a test tube, mixed well, left for 2-3 minutes. Read the test result within 5 minutes using the Nyco Card Reader.

2.13.3. Creatinine level

Creatinine level was estimated by colorimetric method with deproteinization (Bartels et al, 1971) using a kit (Human, Germany). Plasma creatinine in alkaline solution reacts with pirate to form a colored complex, when mixed with kit reagent, left for 20 minutes. It was read at 520 nm against blank.

Reagents

Initial Concentration of solution

1. Standard	2mg /dl 177µmol/l
2. Picric acid	35mmol/l
3. Sodium hydroxide	1.6mol/l
4. TA651 trihloroacetic acid (TCA)	1.2mol/l

Preparation of reagent mixture

The solutions 2 and 3 were mixed in 1:1 ratio which was stable for few hours at 15 to 25^0 C when stored in a dark bottle.Deproteinization: plasma sample was pipette into the centrifuged tubes with TCA. Using a glas-rod to evenly mix, precipitates werewdispersed, this was then centrifuged at 2500 rpm for 10 minutes. Supernatant was poured off, and plasma sample and TCA were mixed in a 1:1ratio.

Assay procedure

Wave length	500-550(Hg546nm)
Spectrophotometer	520nm
Cuvette	1cm light path
Temperature	25^0 C
Measurement	against blank

Pipette into test tubes

	Blank	Standard	Plasma sample
Distilled water	0.5 ml	-	-
Solution 1	-	0.5ml	-
TCA	0.5 ml	0.5ml	-
Supernatant			1ml
Reagent Mixture	1ml	1ml	1ml

Mix, let Standard for was mixed for 20minutes at 25^0C, absorbance of sample (A_{sample}) and standard (A_{sample}) was read against blank.

Calculation: Concentration of creatinine in serum= ($A_{sample}/A_{standard}$) ×2 mg/dl

2.13.4. Micro albumin Colorimetric test BCG-method

Micoalbuminwas measured by colorimetric BCG-method (Rod key et al, 1964) using a kit (Human, Germany).

The kit contained

color reagent	1×1000ml
Citrate buffer (P^H4.2)	30mmol/l
Bromocresol green	260µmol/l
standard	1×3ml
Albumin	40g/l
Sodium azide	0.095%

10µl plasma was mixed with 1000 µl reagent for 5 min at 25^{0}C and absorbance was read at 546 nm.

Calculation

$$\text{Plasma C} = 4 \times \frac{\Delta A_{sample}}{\Delta A_{STD}} [g/dl]$$

$$C = 40 \times \frac{\Delta A \text{ sample}}{\Delta A_{STD}} [g/dl]$$

2.13.5. Analysis of plasma glutamic pyruvic transaminase or SGPT

Plasma SGPT level was estimated by Liquiuv method, (Clin.Chim.et al, 1980) using an Alanine Aminotransferase (EC2. 6.1.2) of Human, Germany.

The kit contained

TRIS buffer (P^H7.4)	125mmol/l
L-alanine	625mmol/l
LDH	≥1.5kU/l
SubstrateZ-Oxoglutarate	75mmol/l
NADH	0.9mmo/l
Sodium azide	0.095%

Procedure for analysis

Content of one bottle substrate was mixed with one bottle buffer. One ml of substrate was mixed to one bottle of buffer, which was stable for 4 weeks at 2- 8^0 C and 5 days at 15-25^{0}C.

200µl plasma+1000µl reagent was incubated at 37^{0}C, absorbance was read at 340 nm, read absorbance after 1 minute, and again exactly after 1,2 and 3 minutes.

Calculation

For ΔA /min within 0.06-0.08 (Hg 365) or 0.12-0.16 (Hg 334nm, 340nm) use only measurements from the first 2 minutes for calculation.

Conversion factor from traditional unit (U/l) in SL –units (Kat/l)

2.13.6. Analysis of plasma glutamic-oxaloacetic transaminase (SGOT or AST)

Plasma SGPT level was estimated by Liquiuv method, (Clin.Chim.et al, 1980) using an Alanine Aminotransferase (EC2. 6.1.2) of Human, Germany.

The kit contained

TRIS buffer (P^H7.4)	100 mmol/l
L-alanine	300 mmol/l
LDH	≥1.5kU/l
MDH	≥0.75kU/l
SubstrateZ-Oxoglutarate	60mmol/l
NADH	0.9mmo/l
Sodium azide	0.095%

Procedure for analysis

One bottle content of substrate was mixed with one bottle buffer. One ml of substrate was mixed to one bottle of buffer, which were is stable for 4 weeks at 2- 8^0 C and 5 days at 15-25^{0}C.

200µl plasma+1000µl reagent was incubated at 37^{0}C, absorbance was read at 340 nm, read absorbance after 1 minute, and again exactly after 1,2 and 3 minutes.

Calculation

For ΔA /min within 0.06-0.08 (Hg 365) or 0.12-0.16 (Hg 334nm, 340nm) use only measurements from the first 2 minutes for calculation.

Conversion factor from traditional unit (U/l) in SL –units (Kat/l)

2.13.7. Estimation of plasma triglyceride

Plasma triglyceride was estimated by enzymatic colorimetric (GPO-PAD) method (Tietz et al, 1990) using kit (Human, Germany.Randox biochemical, UK.

Sample triglyceride incubated with a lipoprotein lipase liberates glycerol and fatty acids. Glycerol is converted to glycerol -3-phosphate by glycerol kinase and ATP. Glycerol phosphate oxidized to dihydroxy acetone phosphate by glycerol phosphate oxidase in the presence of peroxidase, hydrogen peroxidase oxidizes the chromogen

4-aminophenazone/N-ethyl-N-(3-sulphopropyl)-m-anisidine to a violet colored compound. The former hydrogen peroxide is detected by a chromogenic oxygen acceptor, phenolampyrone, in the presence of peroxidase (POD).The red quinone formed is proportional to the amount of triglyceride present in the sample.

Reagents

N-ethyl –N (3-sulphopropyl)-ansedine /Surfactant/Buffer

1A Enzymes /ATP/4-Aminophenazone / potassium

Ferro cyanide

Preparation of working reagents

Thirty ml reagent 1 was added to one vial 1A and mixed gently to dissolve contents. Component and concentration of working solutions:

Buffer	50mmol/1P^{H}7.0
Lipoprotein lipase	≥50 U/ml
Glycerol kinase	≥0.05 U/ml
Glycerol phosphate oxidase	≥2.0 U/ml
Peroxidase	≥0.3 U/ml
Adenosine 5-triphosphate	0.7mmol/l
4-aminophenazone	1.0mmol/l
Potassium ferrocyanide	7.0µmol/l
Magnesium salts	0.6mol/l
N-ethyl –N-(3-sulphopropyl)-m-anisidine	1.2mmol/l
Surfactant stabilizers	2.0g/l

Procedure

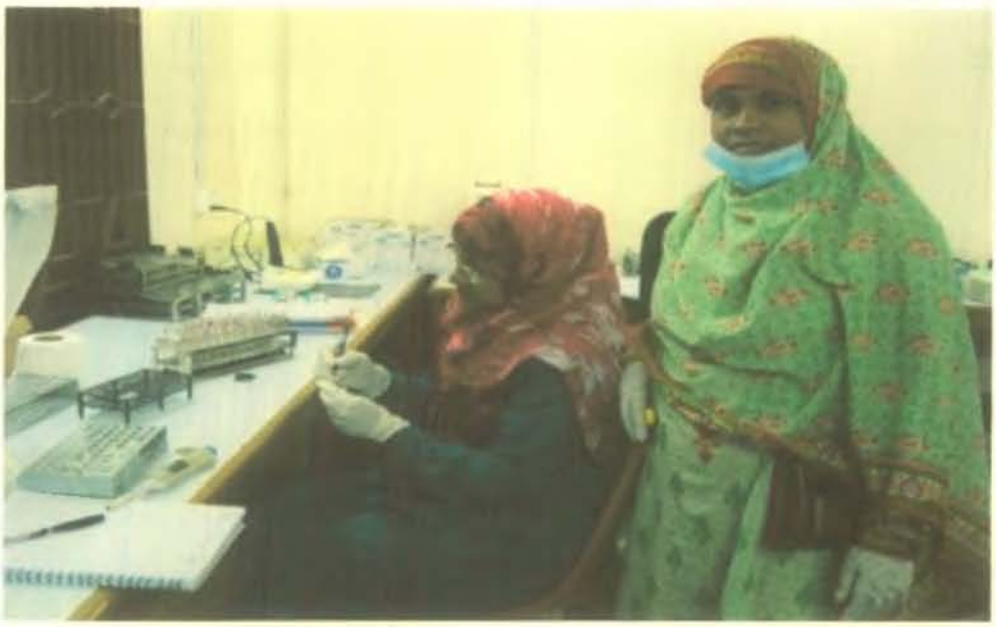
Series of standard triglyceride solution (0, 25, 50,100 and 150mg/dl) were prepared by diluting a stock standard solution of triglyceride (200-mg/dl). Standard triglycerides solutions (50µl) of each concentration were taken in the initial 6 microcells of the plate. Then 5µl plasma was taken in the remaining microcells of the plate and in all wells the working reagent (200µl) was added.

The mixture was then incubated for 5 minutes at 37^{0}C and then absorbance of the solution was measured at 490 nm with micro plate reader. Two parallel experiments were carried out for each sample. Thus a calibration curve was obtained for the absorbance vs. concentrations of the standard solutions. On the basis of the calibration curve, the unknown concentrations of triglyceride in serum were

measured maintain the same mixing and incubation conditions as for the standard solutions. The standard curve was drawn on every experimental day.

Calculation of results

Concentration was calculated by using kinetic-calculation program for micro well plate Reader.



Picture 5: Researchers working in lab

2.13.8. Estimation of plasma total cholesterol

Estimation of serum total cholesterol was done by enzymatic endpoint (CHOD-PAP) method (RandoxLab.UK), using auto analyzer (Tietz et al, 1990)

Free cholesterol and cholesterol released from its esters on enzymatic hydrolysis are oxidized enzymatically.The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

2.13.9. Estimation of HDL, LDL, VLDL

Serum High Density Lipoprotein (HDL) was done by enzymatic colorimetric GPO-PAP method (RandoxLab.UK) using Auto analyzer (Tietz et al, 1990).

High Density Lipoproteins are separated from chylomicrons, VLDL and LDL by the addition of precipitating reagent (phosphotungstic - magnesium chloride) to plasma. After centrifugation, the cholesterol contents of HDL fraction which remains in the supernatant are determined by the enzymatic colorimetric method using total cholesterol, CHOD-PAP reagent.

Precipitation reaction

200 ml plasma was added to 500µl precipitating reagent, mixed well by gentle shaking of tubes. The reaction tubes were left room temperature for 10min and then centrifuged at 4000rpm for 10 minutes. The supernatant separated was taken in fresh tube. Samples were ready for estimation of HDL.

Laboratory technique

The Auto lab Unit was calibrated for the specific test before starting of assay using standard. Serum samples were taken into sample cups and placed in the cup holder. Reagents were taken into reagent containers and placed into specific reagent slot. The Auto lab was programmed for the estimation of glucose, triglyceride, total cholesterol and HDL-cholesterol.ID was entered and the unit was set run. We obtained results and checked the value of the controls to ensure quality controls of tests.

Serum LDL cholesterol level was calculated by Fried Wald formula
Formula: LDL-Cho l= T Chol-(1/5TG+HDL-Chol).

2.13.10. Estimation of plasma Insulin

Plasma Insulin was estimated by Enzyme- linked sandwich method (Flier kahn et al, 1979) was used to estimate plasma Insulin level of DRG, Germany.

The insulin kit is a solid phase enzyme –linked immunosorbent assay (ELIZA) based on sandwich principle. The micro titer wells are coated with a monoclonal antibody directed towards a unique antigenic site on the insulin molecule.
An aliquot of plasma sample containing endogenous insulin is incubated in the coated well with enzyme conjugate, which is an anti-insulin antibody conjugated with Biotin. After incubation the unbound conjugate is washed off. During the second incubation step streptavidin peroxidase enzyme complex binds to the biotin-anti-insulin antibody. The amount of bound HRP complex is proportional to the concentration of insulin in the sample.
Having added the substrate solution, the intensity of colour developed is proportional to the concentration of insulin in the patient sample.

Materials

A micro titer plate calibrated reader (450 ±10nm)
Calibrated variable precision micropipettes
Absorbent paper.
Distilled or deionized water.
Timer
Graph paper or software for data reduction.

Reagents provided

1. Micro titer wells, 12×8(break apart) strips, 96 wells coated with anti-insulin antibody (monoclonal)
2. Zero standard, 1vial, 3mL, ready to use 0μlU/ml, containing non-mercury preservative
3. Standard (Standard 1-5), 5vials, 1mL, ready to use, Conc: 6.25-12.5-25-50 100 μlU /mLConversion: μl U /ml × 0.0433= ng /mL, ng /mL ×23.09 = μl U /mL. The standards are calibrated against international WHO approved Reference material NIBSC66/304; coontaining non-mercury preservative
4. Enzyme Conjugate, 1 vial, 5mL, ready to use, Mouse monoclonal anti-insulin conjugated to biotin. Contains non-mercury preservative.
5. Enzyme complex, 1 vial, 7 mL, ready to use, streptavidin-HRP complexContains non-mercury preservative.
6. Substrate solution, 1 vial, 14 mL, ready to use, Tetraethylbenzidine (TMB)
7. Stop solution, 1 vial, 14 mL, ready to use, containing $0.5MH_2SO_4$.
8. Wash solution, 1 vial, 30 mL (40×concentrated).

Test Procedure

Each run ncluded a standard curve

1. the desired number of Micro titter wells in the frame holder was secured,
2. 25μL of each standard, control and plasma with new disposable tips in to appropriate wells was dispensed,
3. 25μL enzyme conjugate in to each well was added,
4. mixed thoroughly mix for 10seconds,
5. incubated for 30 minutes at room temperature,
6. Briskly shake out the contents of the wells was made,
7. rinsed the wells 3 times with diluted wash solution (400μL per well), striked the wells sharply on absorbent paper to remove residual droplets,

The sensitivity and precision of this assay was found to be markedly influenced by the correct performance of the washing procedure.

8. added 50 μL of Enzyme complex to each well,
9. incubated for 30monutes at room temperature,
10. briskly shaked out the contents of the wells,
11. rinsed the wells 3 times with diluted wash solution (400μL per well), strikd the wells sharply on absorbent paper to remove residual droplets,
12. added 50 μL of Substrate solution to each well,
13. incubated for 30monutes at room temperature,

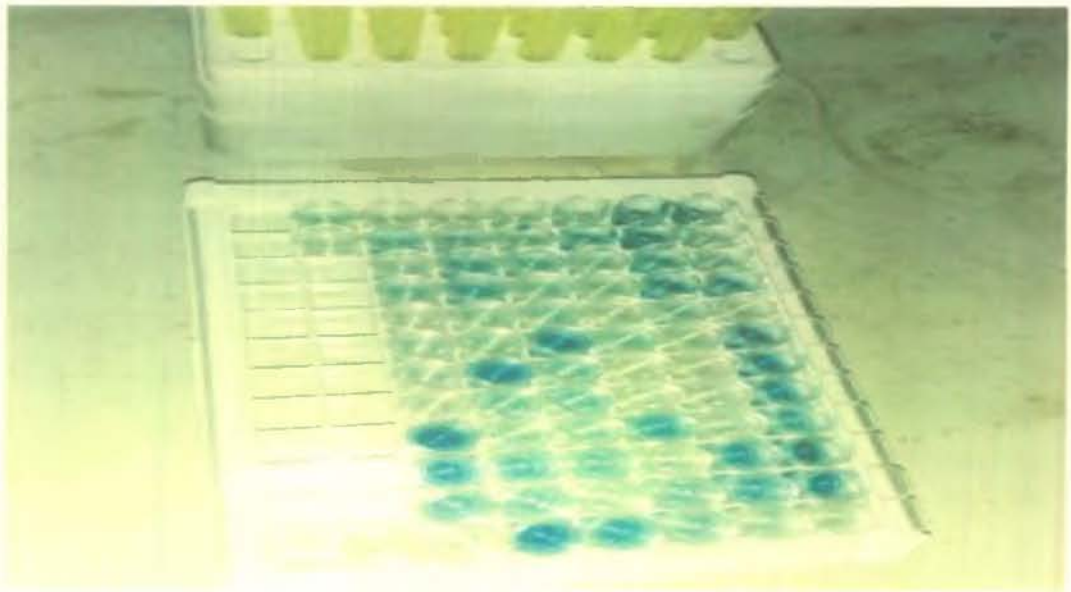
14. stopped the enzymatic reaction by adding 50 µL of stop solution to each well, and
15. the absorbance (OD) of each well read at 450± 10 nm with a micro titer plate reader.Ewells were read within 10minutes after adding the stop solution.

Calculation of results

1. Calculated the average absorbance values for each set of standards, controls and patient samples,
2. Using linear graph paper, constructed a standard curve by plotting the mean a absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis,
3. Using the mean absorbance value for each sample determined the corresponding concentration from the standard curve,
4. The results in the instructions for use have been calculated automatically using a 4-parameter curve fit. Other data reduction functions may give slightly different results,
5. The concentration of the samples could be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as ≥100 µLU/ mL. For the calculation of the concentrations this dilution factor has to be taken in to account.

Expected Normal values

ELISA the following values are observed: 2 µl/U mL to 25 µl/U mL



Picture 6: Analysis of plasma insulin

2.13.11. Estimation of plasma Zinc

Flame Atomic Absorption Spectroscopy (FAAS) was used in determination of plasma zinc concentration. It is an easy and fast method with high sensitivity mainly for elements like Cu and Cr, but difficulties may arise as a result of chemical and spectral interventions. Radiation of a specific wavelength is chosen by using hollow cathode lamp through the sample is atomized. Concentration of the analyzed element is measured by the amount of absorbed radiation. The most courant gas mixtures used are air/acetylene and nitrous-oxide/acetylene. Background correction can be achieved with a deuterium lamp though several drawbacks subsequently occur. When absorbance becomes higher than 0.5 to 1 then the non-linearity of the standard curve is a major disadvantage of the AAS technique. The relative standard deviations are between 0.3 and 1% for absorbance of 0.1 to 0.2. Detection limits for flame AAS vary immensely from 1-5 ppb (e.g. Ca, Cd, and Cu) to more than 1000 ppb. Some elements like B, C, and Br cannot be measured at all by AAS.

Serum zinc levels of human were estimated by atomic absorption spectrophotometric method (I>Perkin Elmer, Atomic Absorption Spectrometer AAnalyst, 200, version-8.0, copy right -2013) as described by (Hossain et al, 2007).

Calibration of standard curve

Calibration curve was obtained using standard samples (containing 0.2, 0.4, 0.8, 1and 1.6 mg/L for copper; 0.1, 0.2, 0.5, 1, 2 and 3 mg/L for iron; and 0.1, 0.2, 0.4 and0.8 mg/L for zinc). All standard solutions were dissolved in nano pure water.Standards were aspirated through nebulizer and the absorbance was measured witha blank as reference, read in the atomic absorption spectrophotometer at 213.9 nm wavelengths for the zinc. Blanksample was Nitric acid (HNO3) without any mineral. A software package for thespectrophotometer constructed calibration curves for zinc by plotting absorbance against the concentrations. It gave straight lines. The correlation coefficient was found for zinc 0.996.

Preparation of standard solutions

Standard solutions containing 10, 20, 40, 50, 80, 100, 160, 200 and 300 µl for zinc were prepared in 100 ml nano pure water in a volumetric flux for each dilution.

Procedure for plasma analysis

Plasma was centrifuged at 3000rpm for 10 minutes to make a clear supernatant. A volume of 150 µl plasmawas collected in eppendrof tube for the analysis of zinc which was diluted 10x with nano pure waterand vortexes for half minutes. Within two hour of mixing, absorbances were read at 213.9 nm wavelengths for the zinc in the atomic absorption spectrophotometer. Machine software calibrated standard curve with standard preparation at every 10 sample interval. Specific hollow cathode lampwere used to analyze the sample for zinc. The instrument minimum detected limit of 0.01 mg/L for zinc.

Calculation

Concentration of zinc was calculated as follows

Concentration, C = Absorbance of sample × dilution factor (10 fold) × F µmole/L

F= 0.1530 was factor for zinc.

Picture 7: Atomic energy spectrometer-AA240 for zinc analysis

2.13.12. Determination of plasma MDA level

Plasma level of MDA usually measured as thiobarbituric acid reactive substances (TBARS) or lipid peroxides (Buge, Aust et al, 1972), which is the most widely produced biologically relevant free radical reaction (Samir, El-kholy et al, 1999). After mixing of 100 µL serum with 900 µL of 0.9% saline solution; 2 mL of freshly prepared thiobarbituric acid (TBA) reagent and 30 µL of 50 mMbutylatedhydroxytolune (BHT) were added. The mixture was then incubated at 60°C for 15 min. For cooling the sample it was kept in ice for another 5 minutes. The sample was then centrifuged at 5000 rpm for 10 minutes. The absorbance of the supernatant was measured spectrophotometrically at 535 nm using 1,1,3,3 tetraethoxypropane as standard.

Picture 8: Analytical report observing

Chapter three

Results

3. Results

This study investigated effect of therapeutic zinc supplementation to type 2 diabetics to lower the blood glucose. In doing this, sociodemography, nutritional status, effect of zinc on insulin, fasting glucose, HbA1c, stress biomarker, effect of diet restriction and physical activity on blood glucose were addressed.

3.1. Sociodemography

Majority of the respondents zinc group (65%) was female and placebo group (53.3%) was male. while the mean age range of zinc group 48.73±7.4 years and placebo group 50.27±7.97 years. It was matched, did not have significant change ($p \geq 0.05$). Differences between groups means was determined by independent sample t-test. Majority (100%) were married zinc group and placebo group were (96.7%) married. Illiterate and (33.3%) of zinc group were unemployed (63.3%). Illiterate and (36.7%) of placebo group were employed (36.7%). The results were expressed as mean ±SD.

Table-4: Socio-demographic profilesof type-2 Diabetic Patients

Variables	Zinc group		Placebo group		Significance
	n (%)	Mean± SD	n (%)	Mean ±SD	
Age in years					
35-40	12 (20)	48.73±7.4	1 (3.3)	50.27±7.97	t= -0.981,
41-50	28 (46.7)	35-75	18 (60)	36-69	df=88
≥51	20 (33.3)		11 (36.7)		p=0.329
Marital status					
Married	60 (100)		29 (96.7)		
Unmarried	00 (0)		01 (3.3)		
Education					
Can sign/Read & write	19 (31.7)		1 (3.3)		
SSC	11 (18.3)		11 (36.7)		
HSC	20 (33.3)		11 (36.7)		
Graduate & above	10 (16.7)		07 (23.3)		
Occupation					
Household works	38 (63.3)		10 (33.3)		
Service	13 (21.7)		11 (36.7)		
Others*	09 (15.0)		09 (30.0)		
Monthly income BDT					
2000-4500	15 (25.0)	6220.85	4 (13.3)	6099.6±1634.3	t=.243,df=88
4501-7000	22 (36.7)	±2466.3	16 (53.3)	3333.33-11,250	P=.808
≥7001	23 (38.3)	2142.9-15,000	10 (33.3)		
Gender					
Male	21 (35.0)		16 (53.3)		
Female	39 (65.0)		14 (46.7)		

Significance p<0.05

Others=Agriculture, NGO worker, Motor driver and business

3.2. Nutritional status

Most of the diabetic subjects were found to be obese in both of the zinc and placebo groups (table 5). Pearson's correlation analysis was performed to find out the correlation of BMI and socioeconomic factor of type 2 diabetic patients. A multiple regression analysis and one-way analysis of variance (ANOVA) were also performed to determine the extent of involvement of socioeconomic factors that no effects of type 2 diabetic patients.

Table-5: Nutritional status

BMI(Kg/m^2)	Zinc group		Placebo group		Significance P<0.05
	n (%)	Mean± SD	n (%)	Mean ±SD	
18.5-24.9 (normal)	19 (31.7)		07 (23.3)		
25-29.9 (overweight)	28 (46.7)	27.20±3.3	21 (70.0)	26.9±2.2	t=0.507, df=88 p= 0.614
Obese-I (30-34.9)	12 (20.0)		02 (6.7)		
Obese-II (35-39.9)	01 (1.7)		00 (0.0)		

3.3. Changes in fasting plasma glucose level

Zinc and placebo supplementation was given to the zinc and placebo groups of diabetic patients. In zinc group, fasting blood glucose level in baseline, 1st followUp, 2nd followup and 3rd followup and 3rd follow up or endline were 14.2±3.9, 13.2±3.2, 12.1±2.9 and 9.4±1.7 mmol/L respectively, while in the placebo diabetes, it were 16.0±3.4,14.3±2.8,15.0±3.2 and 3.3±2.1 mmol/L respectively (table 6).

The fasting blood glucose were found to be reduced significantly in zinc group in endline ($p< 0.001$). It was remain unaltered in the placebo group.

Table 6: Changes in fasting plasma glucose levels in type 2 diabetes undergoing zinc Supplementation

Fasting glucose in mmol/L	Zinc group		Placebo group		Significance
	n (%)	Mean± SD	n (%)	Mean ±SD	
Baseline (n=183)[a] 6.9-13.0 13.0-19.0 ≥19.1	77 (42.1) 84 (45.9) 22 (12.0)	14.2±3.9	n=64 17 (26.6) 30 (46.9) 17 (26.6)	14.6±3.4	t= -1.71, p=0.443
1st follow up (n=157)[b] 6.9-13.0 13.0-19.0 ≥19.1	83 (52.9) 66 (42.0) 08 (5.1)	13.2±3.2	n=58 18 (31.0) 39 (67.3) 01 (1.7)	14.3±2.8	t= -2.06, p=0.03
2nd follow up (n=101)[c] 6.9-13.0 13.0-19.0 ≥19.1	68 (67.3) 31 (30.7) 02 (2.0)	12.1±2.9	n=41 16 (39.0) 17 (41.5) 08 (19.5)	15.0±3.2	t= -3.96, p=0.000
3rd follow up (n=60)[d] 6.9-13.0 13.0-19.0 ≥19.1	58 (96.7) 02 (3.3) 00 (00)	9.4±1.7	n=30 11 (36.7) 18 (60.0) 01 (3.3)	13.3±2.1	t= -9.986, p=0.000

Significance p<0.05

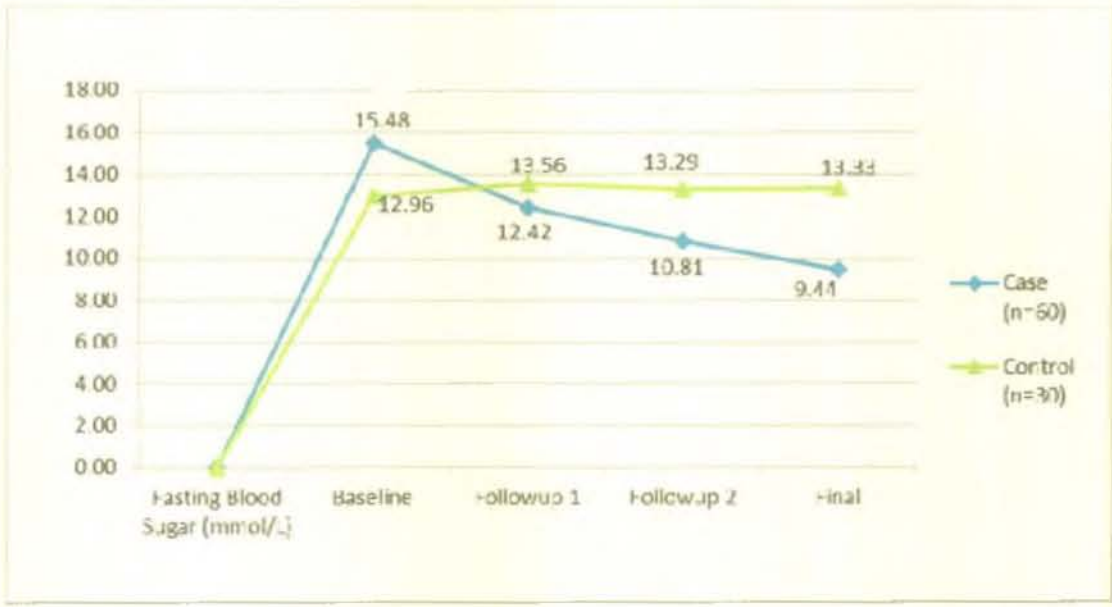


Figure 1. Fasting palsma glucose level (mmol/L) in zinc and placebo groups during intervention

3.4. Multiple comparison tests

Multiple comparison tests showed that changes in fasting glucose in zinc group at baseline,1st follow up,2nd follow up and 3rd follow up or endline was significant (p<0.000). Crosstab result was significant in zinc group, but there had no significant (p<0.011) in the placebo group (table 7).

Table 7: Crosstab analysis for comparison of fasting blood glucose

Zinc group	[a]Baseline (n=183)n(%)	[b]Followup-1(n=157) n (%)	[c]Followup-2 (n=101) n (%)	[d]Follow up-3 (n=60) (%)
6.9-13.0	77 (42.1)	83 (52.9)	68 (67.3)	58 (96.7)
13.0-19.0	84 (45.9)	66 (42.0)	31 (30.7)	02 (3.3)
≥19.1	22 (12.0)	08 (5.1)	02 (2.0)	00 (00)
Mean± SD	14.3±3.9	13.2±3.2	12.1±2.9	9.8±2.2
Placebo group	[a]Baseline (n=64)n(%)	[b]Followup-1(n=58) n (%)	[c]Follow up-2 (n=41) n (%)	[d]Follow up-3 (n=30)n (%)
6.9-13.0	17 (26.6)	18 (31.0)	16 (39.0)	11 (36.7)
13.0-19.0	30 (46.9)	39 (67.3)	17 (41.5)	18 (60.0)
≥19.1	17 (26.6)	01 (1.7)	08 (19.5)	01 (3.3)
Mean± SD	14.9±2.7	14.2±2.7	15.3±3.2	13.3±2.1

Zinc group: ANOVA TUKEY	$F^{abcd}(3,497)=29.448$ P=.000 $P^{ab}=0.047$, $P^{ac}=0.000$, $P^{ad}=0.000$, $P^{bc}=0.016$, $P^{bd}=0.000$, $P^{cd}=0.000$	$F^{bcd}(2,315)=29.139$ P=.000 $P^{bc}=0.003$, $P^{bd}=0.000$, $P^{cd}=0.000$
Placebo group: ANOVA TUKEY	$F^{abcd}(3,189)=3.773$ P=.012 $P^{ab}=0.421$, $P^{ac}=0.900$, $P^{ad}=0.047$ $P^{bc}=0.178$, $P^{bd}=0.537$, $P^{cd}=0.017$	$F^{bcd}(2,315)=4.634$ P=.011 $P^{bc}=0.109$, $P^{bd}=0.377$, $P^{cd}=0.010$

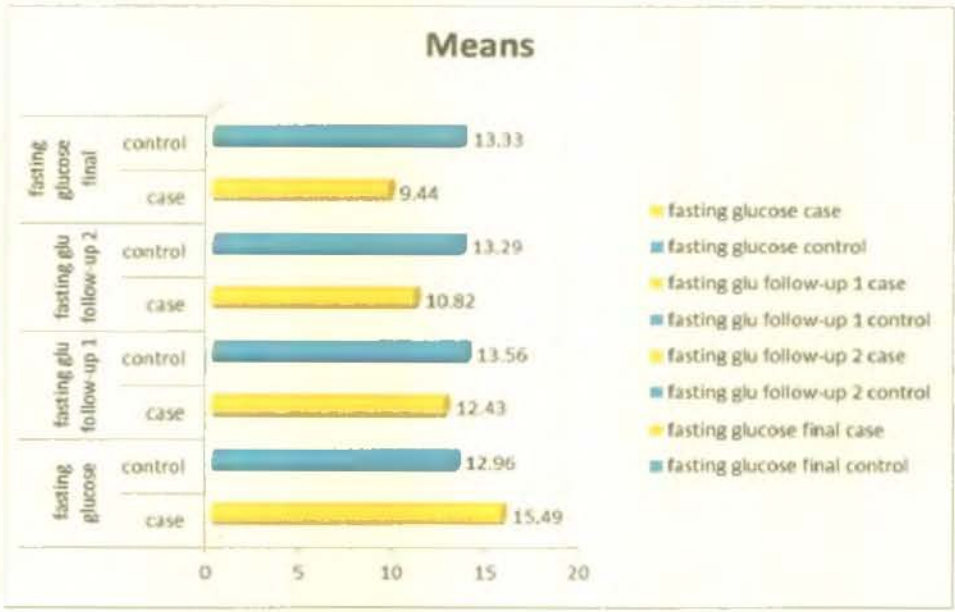


Figure2: Fasting plasma glucose level in zinc and placebo groups during intervention

3.5. Changes in glycated haemoglobulin

Table 8 showed that glycalated Hb in the zinc group at baseline was 13.4±3.9mmol/L and at endline or after 3 months, it was 10.9±1.9 mmol/L, and in the placebo group, it were 13.4±3.9 and 10.9±1.9 mmol/L. HbA1c in the zinc supplemented group had reduced significantly ($p< 0.04$); but the HbA1c change in placebo group was positively increase, and the increase was significantly ($p=0.000$) high.

Table 8: Changes in glycated Hb (HbA1c)

HbA1c	Zinc group		Placebo group		Significance
Baseline n=183	n (%)	Mean± SD	n (%) n=64	Mean± SD	
7.80-13.0	90 (53.3)		14 (22.2)		
13.1-18.0	64 (37.9)	13.4±3.9	49 (77.8)	14.0±1.7	t=-2.09, P=0.04
≥18.1	15 (8.9)		00 (00)		
Endline n=60			n=30		
7.80-13.0	53 (84.1)		06 (20.0)		
13.1-18.0	10 (15.9)	10.9±1.9	23 (76.7)	14.7±2.3	t=-6.09, P=0.000
≥18.1	00 (00)		01 (3.3)		

Significance p<0.05

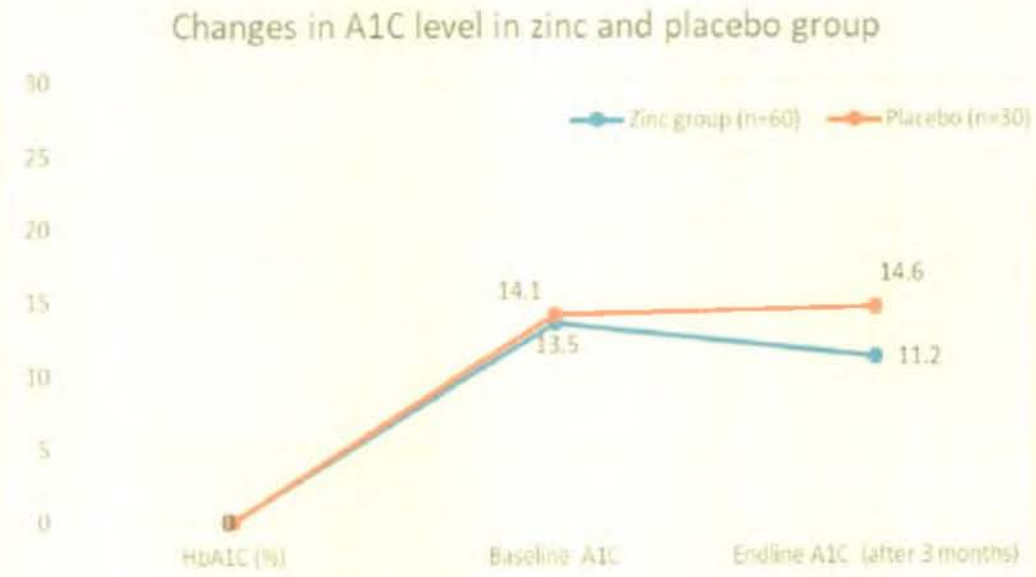


Figure 3: Change HbA1c during intervention

3.6. Analysis of biochemical profile

Plasma creatinine, micro albumin and SGPT, SGOT and lipid profilesof the study zinc group were analysed using standard reference analytical methods. An attempt to find out the association or in fluence of the biochemical parameters with sociodemography and nutritional status was also taken. Zinc supplementation made a significant ($p<0.05$) reduction of most of the biochemicals, except plasma SGPT, cretonne, HDL ($p>0.05$). Table 9 presents the biochemical profilesof the type 2 diabetes, most of which were found to be near to normal values, except the fasting blood glucose (15.5mmol/L).

Chi-square analysis indicated that socio demography and nutritional status did not have any effect or association on or with the biochemical indices (table 10, 11). However, it was seen that HDL and LDL had apparently influence by the education level, and urine micro-albumin was also influenced by gender. The sociodemography or nutritional status did not show any influence on the fasting blood glucose level in either baseline or end line value (table 12).

Table 9: Biochemical profilesof type-2 Diabetes undergoing zinc supplementation

Biochemical parameters (n=60)	**Baseline**	**Endline**	**p-value#**
SGOT (U/L)	46.1±11.3	46.7±11.4	p=0.447
SGPT (U/L)	41.5±10.7	39.2±9.3	p=0.005*
Serum creatinine (mg/dl)	1.22±0.47	1.19±0.46	p=0.174
Urine microalbumin (µg/mg)	38.4±8.7	31.3±7.7	p=0.000*
LDL(mg/dl)	163.4±35.1	151.9±31.9	p=0.000*
HDL(mg/dl)	37.1±5.6	37.7±5.2	p=0.178
Cholesterol(mg/dl)	206.7±31.2	192.8±26.4	p=0.000*
Triglyceride(mg/dl)	227.9±54.6	220.4±49.4	p=0.003*
Fasting blood glucose(mmol/L)	15.5±4.7	9.4±1.7	p=0.000*

Paired sample t-test P<0.05

3.7. Analysis of of plasma zinc, insulin and malondialdehyde

Plasma zinc, insulin and biomarker malondialdehyde were analysed to assess the level of these components in the zinc supplemented and placebo groups. Flame Atomic Absorption Spectroscopy (FAAS) method was employed in estimation of plasma zinc value, enzyme- linked sandwich method was used to estimate plasma Insulin level, and thiobarbituric acid reactive substances or lipid peroxides was employed to measure the plasma malondialdehyde content.

3.7.1. Plasma zinc level

Compared to normal plasma zinc value (11.5 to 18.5 µmol/L), the diabetes subjects were found to have zinc deficiency (7.87 µmol/L). Zinc supplementation made a significant (p=0.0001) sharp increase, which stand to 76.25 µmol/L (table 10). In placebo group, this change was significant (p=0.002), but it was very little, only 7.93µmol/L to 9.83µmol/L (figure 3).

3.7.2. Plasma insulin content

Zinc therapy also made a significant (p=0.001) in both ANOVA and t-test. Plasma insulin was gradually increased from 12.03 to 16.26 to 19.56µIU/ml. In case of placebo group, insulin level was also increased from 12.06 to 13.41 µIU/ml (table 11, figure 4), and it was also significant (p=0.001).

3.7.3. Plasma malondialdehyde

Zinc supplementation had shown too significantly (p=0.001, 0.047) reduce plasma malondialdehyde level (table. Zinc reduced the malondialdehyde from baseline 3.30±0.87 to 2.09±1.03µmol/, while in the placebo group, zinc therapy had increased the plasma malondialdehyde from 3.22±0.99 to 4.19µmol/ (table 12, figure 5). The increase by zinc and decrease in placebo were found significant (p=0.00),

Table 10: Plasma zinc level in zinc-supplemented and placebo groups of type II diabetes

Plasma zinc level µmol/L	Zinc supplementation group (zn)				Placebo group (plb)			
	Baseline[a]		End line[b]		Baseline[a]		End line[b]	
	N (%)	Mean ± SD	N (%)	Mean± SD	N (%)	Mean± SD	N (%)	Mean± SD
<9.90 Clinical Deficiency	31(62)	4.96±2.85	5(10)	3.85±2.44	22(75.9)	5.62±3.63	25(51.7%)	5.02±3.91
≤ 10.5 Deficiency	-		-		--		-	
10.5-18.5 Normal range	19(38)	12.61±1.31	2(4)	14.55±0.29	7(24.1)	15.17±0.47	'3(44.%)	14.58±2.34
18.6-22.9, Above normal	-		2(4)	20.16±0.89	--		1(3.4%)	20.07
≥ 23.00 Excess	-		41(82)	90.82±41.96	--		--	
Total	50(100)	7.87±4.44	50(100)	76.25±49.34	29(100)	7.93±5.21	29(100)	9.83±6.04

Statistics (t-test) ZnBE: t=9.97, p = 0.0001 PlbBE: t =3.45, p= 0.002 (BE: baseline endline)

Mauchly's test indicated that the assumption of sphericity had been violated. The sphericity assumed test indicated significant interaction among group: (F 1, 77) =54.120, P<0.0001. Post hoc comparisons between zinc-placebo grps at baseline P= 0.964, and at endline zinc group had significant high insulin P = 0.000.

Plama zinc value: 11.5 to 18.5 µmol/L ref: Young Implementation of SI units for clinical laboratory data. Annals of internal medicine 1987, 106:114-129.

Optimal plasma zinc range: 13.8 t0 22.9µmol/L; ≤ 9.9 µmol/L Clinical deficiency; < 10.7 µmol/L Deficiency; ≥18.5 or 23 excess values.

Zinc defiency is common among type 2 diabetic patients (Daradkeh et al, 2014).

Physical exercise have been reported to increase serum zinc concentration (Chu and Samman, Vitam Miner 2014, 3:3; Chu et al, 2017).

Physical exercise can disrupt cellular structures, which releases proteins and zinc from myocytes. Two genes ZnT protein and Zip family regulate the zinc influx and efflux in cells that maintains zinc haemotasis; ZnT up regulates and increase zinc level by reducing intracellular zinc (Driskell, 2009).

Diabetic diet contains proteins and vegetables, which are suggested to increase zinc content in body (Saunder et al, 2012; Sloup et al, 2017)

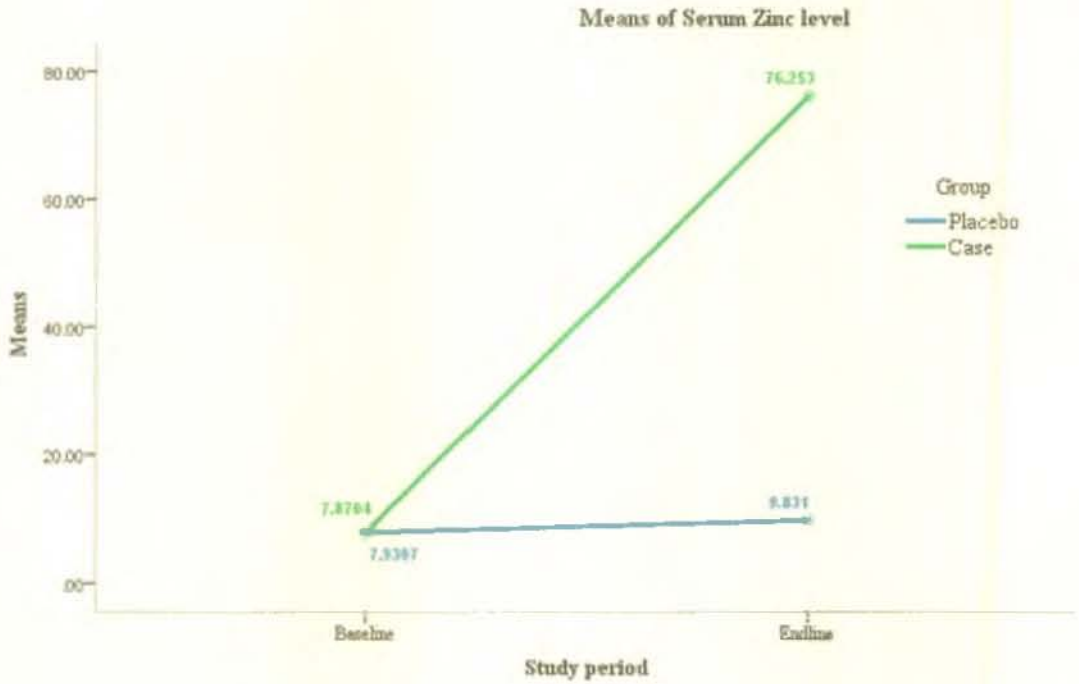


Figure 4: Plasma zinc content in zinc supplemented and placebo group of diabetes

Table 11: Plasma insulin value in zinc-supplemented and placebo groups in type II diabetes

Plasma insulin level µIU/ml	Zinc supplementation group (zn)				Placebo group (plb)			
	Baseline[a]	1st Fup[b]	2nd Fup[c]	3rd Fup/EL[d]	Baseline[a]	1st Fup[b]	2nd Fup[c]	3rd Fup/EL[d]
< 12.0	28(56%)	2(4%)	2(4%)	-	9(45%)	11(55%)	18(90%)	5(25%)
Mean ± SD	10.31±0.8	11.42±0.40	11.04±0.00		10.80±.546	10.82±0.59	8.65±1.98	10.74±1.02
12.01-13.0	9(18%)	2(4%)	2(4%)	-	8(40%)	2(10%)	1(5%)	5(25%)
Mean ± SD	12.62±0.28	12.26±0.10	12.52±0.00		12.56±.285	12.71±0.00	12.76	12.51±0.25
>13.01	13(26%)	46(92 %)	46(92%)	50(100%)	3(15%)	7(35%)	1(5%)	10(50%)
Mean ± SD	15.33±2.57	16.74±2.75	16.65±1.88	19.56±4.72	14.50±.192	13.93±0.80	13.95	15.18±2.19
Total	50(100%)	50(100%)	50(100%)	50(100%)	20(100%)	20(100%)	20(100%)	20(100%)
	12.03±2.59	16.35±2.96	16.26±2.25	19.56±4.71	12.06±1.40	12.10±1.62	9.12±2.37	13.41±2.50

Statistics **Zinc supplemented group (Zn)**
ANOVA abcd: F-47.582, p= 0.00; bcd: F=14.673, p = 0.00
t-test ab: p = 0.0001, mean diff (95% CI) -4.323*(-5.447, -3.198)
bc: p = 1.00, mean diff (95% CI) .091 (-1.394,1.575)
cd: p= 0.0001, mean diff (95% CI) -3.292*(-5.190,-1.395)

Placebo (plb)
ANOVA abcd: F (16.537), p(0.00) F= 20.453, p = 0.00
t-test ab: p = 1.00, mean diff (95% CI) -.042 (-1.533, 1.449)
bc: p = 1.00, mean diff (95% CI) 2.981* (1.157, 4.804)
cd: p= 0.0001, mean diff (95% CI) -4.283*(-6.589 -1.977)

Mauchly's test indicated that the assumption of sphericity had been violated. Greenhouse-Geisser tests showed a significant interaction between zinc and placebo groups: (F 2.22, 161.16) =17.29, (P<0.0001). Post hoc comparisons between zinc-placebo grps at baseline: P= 0.964, at endline zinc group had significant high insulin level P=0.000.

Plasma insulin level: 5-20 µIU/ml normal mean value: ~ 12.5 µIU/ml

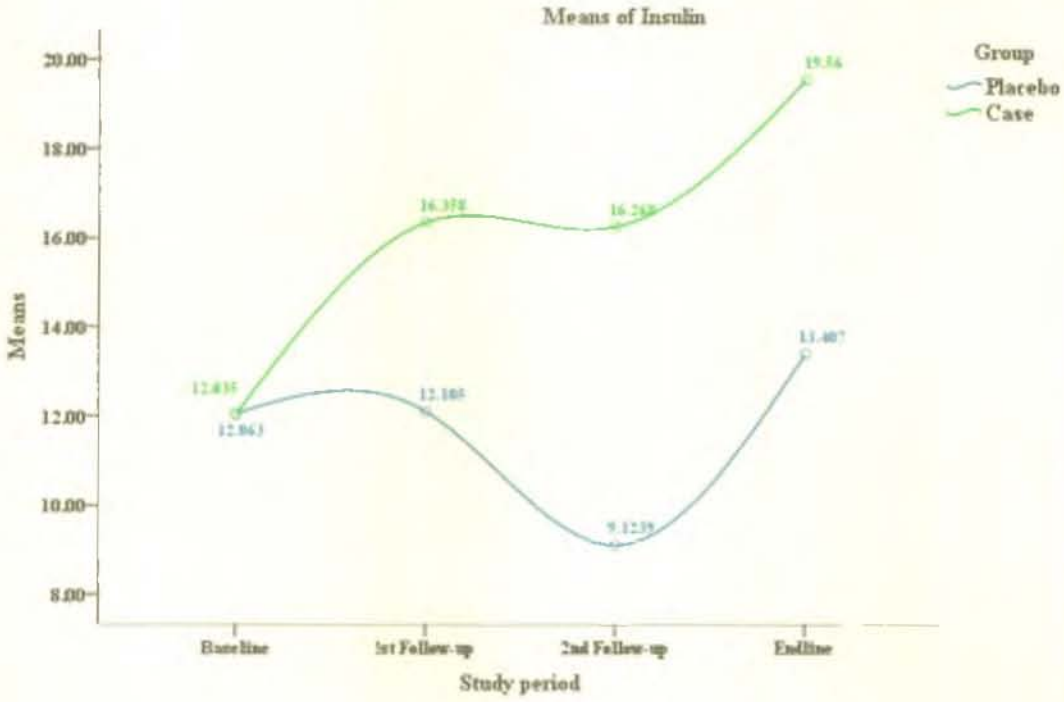


Figure 5: Plasma insulin level of zinc supplemented and placebo group of diabetes

Table 12: Plasma malondialdehyde level in zinc-supplemented and placebo groups in type II diabetes

Plasma MDA	Zinc supplementation group (zn)				Placebo group (plb)			
	Baseline[a]	1st Fup[b]	2nd Fup[c]	3rdFup/EL[d]	Baseline[a]	1st Fup[b]	2nd Fup[c]	3rdFup/EL[d]
< 3.0	20(34.5)	33(73.3)	34(75.6)	27(81.8)	13(43.3%)	-	-	9(36)
Mean±SD	2.44±0.66	2.15±0.43	1.75±0.63	1.69±0.54	2.31±0.58	-	-	2.66±0.23
~ 3.0-3.9	27(46.6)	6(13.3)	7(15.6)	5(15.2)	10(33.3%)	11(44%)	1(4)	8(32)
Mean±SD	3.46±0.30	3.32±0.17	3.47±0.22	3.49±0.25	3.49±0.29	3.37±0.28	3.54	3.31±0.23
>4.0	11(19)	6(13.3)	4(8.9)	1(3)	7(23.3%)	14(56%)	24(96)	8(32)
Mean±SD	4.55±0.43	4.94±0.58	5.65±0.048	5.62	4.50±0.36	4.46±0.53	4.224±0.00	4.17±0.72
Total	58(100)	45(100)	45(100)	33(100)	30(100)	25(100)	25(100)	25(100)
	3.30±0.87	2.67±1.06	2.36±1.32	2.09±1.03	3.22±0.99	3.98±0.70	4.19±0.13	3.35±0.66

Statistics

Zinc supplemented group (zn) ANOVA abcd: F=13.194, p=0.00, bcd= 3.592, p = 0.047
t-test ab: p = 0.032, mean diff (95% CI) 0.675*(.040, 1.310); bc: p = 0.477,
Mean diff (95% CI) 0.521*(-0.288, 1.331) cd: p= 1.00, mean diff (95% CI) .091(-0.347, 0.529)

Placebo (plb) ANOVA F=12.842, p=0.00, bcd=14.785, p =0.00
t-test ab: p = o.023, mean diff (95% CI) -0.860* (-1.631,-0.089); bc: p = 0.813, mean diff (95% CI) -0.210(-0.599, 0.180)
cd: p= 0.0001, mean diff (95% CI) 0.839*(0.475,1.203)

In Mauchly's test, the assumption of sphericity had been violated. The Huynh-Feldt tests indicated a significant interaction between groups between time interval and group (F 2.63, 147.7) =18.067, (P<0.0001). In Post hoc comparisons between the two groups at base line (P= 0.310, at endline, zinc group, there had a significant difference between the two groups at each followup, zinc group have high zinc levels P (0.000).

MDA plasma concentration: 3.34± 0.71µmol/Lref: Dahake HS et al. Int J Res Med Sci. 2016 Nov;4(11):4730-4734

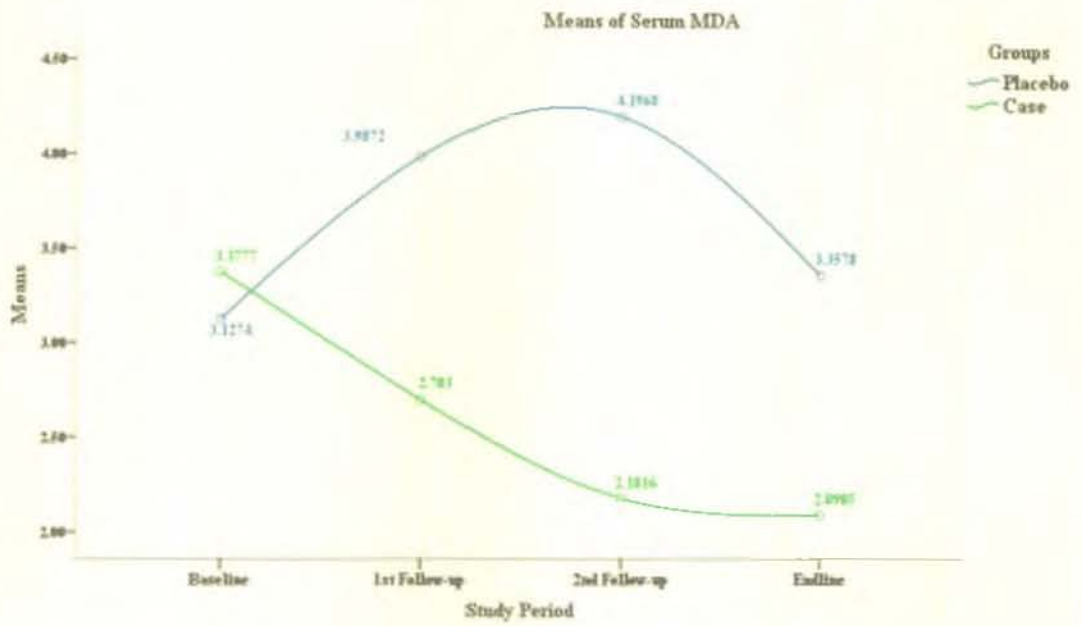


Figure 6: Plama malondialdehyde in zinc supplemewnted and placebo group of diabetes

3.8. Diet restriction and physical activity of diabetic subjects

Inaddition to zinc supplementation, diet restriction and physical activity or exercise were adviced and monitor to diabetic patient for maintenance of blood glucose leve;

In order to reduce and slow insulin need, the diabetic subjects included in this study was adviced and monitored. The diet intake of the subjects and zinc yield from the diet are present in the table 13,14. To improve the insulin synthesisand secretion, diabetic subjects were also suggested to perform physical activity, which is described in the tabe 15.

Table 13: Food consumption patterns of type-2 diabetic patients by food groups and nutrient sources

Nutrients (Food groups)	Nutrient sources	Food names	Baseline consumption (Before diet counseling)		End line consumption (after 3 months)	
			Food weight g Mean± SD	Calorie consumed Kcal	Food weight g Mean± SD	Calorie Consumedcal
Carbohydrate (cereals/grain/tubers)	Plant	Rice	277.0±11.2	1108.0	100.0±0.8	400.0
		Red Wheat	90.1±5.0	360.4	80.0±0.6	320.0
		Potato	54.1±5.0	216.4	65.0±1.8	300.
		Total	421.2±21.2	1684.8	245.0±3.2	1020.0
Protein, vitamins and minerals (Meat/fish/eggs/milk/ Lentils/[a]GLV/NLVs/fruits)	Animal and plant	Beef	07.2±0.1	28.8	05.0±0.2	20.0
		Chicken/Duck	15.2±0.7	60.8	10.0±0.7	40.0
		Eggs	10.1±0.8	40.4	10.0±0.8	40.0
		Fish	65.2±0.5	360.8	60.0±0.5	240.0
		Milk & milk prd	10.2±0.8	40.8	15.0±0.8	80.0
		Lentil	19.4.±0.4	77.6	10.0±0.4	40.0
		GLV	25.2±0.2	100.8	50.0±0.2	400.0
		NLV	110.1±1.1	440.4	150.0±1.1	600.0
		Fruits	25.0± 0.9	100.0	25.0±0.4	102.0
		Total protein	287.6±5.4	-	335.0±5.1	-
Fat	Plant and Animal	Plant Oil	15.3±1.5	137.7	10.0±3.9	90.0
		Animal fat	10.2±7.3	91.8	5.0±5.0	45.0
		Total Fat	25.5±8.8	229.5	15.0±8.9	135.0
Miscellaneous foods			10.0±0.1		10.0±0.1	
Total foods (g) and calorie consumption (Kcal)			824.6±32.5 (g)	3124.7 ± 453.43	603.0±4.8 (g)	2455.0 ±145.36
Frequency of meal/day			Baseline		End line	Statistics
<4			55.0 (33)		35.0 (21)	χ^2=3.61
≥4			45.0 (27)		65.0 (39)	P=0.35

[a]GLV= Green leafy vegetables, NLV=Non leafy vegetables

Table 14: Zinc consumption (µg) from the food intake (g) expressed in Mean± SD by the diabetes subjects

Nutrient	Food source	Food name	Baseline		Endline	
			Food	Zinc	Food	Zinc
Carbohydrate	Plant	Rice	277.0±11.2	45±15	100.0±0.8	16±31
		Red Wheat	90.1±5.0	127±89	80.0±0.6	92±63
		Potato	54.1±5.0	22±42	65.0±1.8	26±0
		Total CHO	421.2±21.2	90±40	245.0±3.2	113 ±94
Protein (also vitamins and minerals	Animal and plant	Beef	07.2±0.1	7±2	05.0±0.2	05±01
		Chicken/Duck	15.2±0.7	12±57	10.0±0.7	8±26
		Eggs	10.1±0.8	10±68	10.0±0.8	10±61
		Fish	65.2±0.5	47±68	60.0±0.5	30±94
		Milk & milk products	10.2±0.8	0±02	15.0±0.8	0±39
Vegetable and fruit	Plant	Lentil	19.4.±0.4	41±63	10.0±0.4	21±89
		GLV	25.2±0.2	12±94	50.0±0.2	32±42
		NLV	110.1±1.1	26±05	150.0±1.1	35± 52
		Fruits	25.0± 0.9	9±36	25.0±0.4	9±45
		Total protein	367.9±2.5	167±95	333.0±1.5	294±49
Fat	Plant and anima	Plant Oil	15.3±1.5	-	10.0±3.9	-
		Animal fat	10.2±7.3	-	5.0±5.0	-
		Total Fat	25.5±8.8	-	15.0±8.9	-

Islam et al, 2012; Shaheen et al, 2014

Table 15: Energy used by Different Physical Activities of Daily Life (1440 minutes/24 hours) by type-2 diabetic patients

Physical activities[a] (n)	PAR or Energy cost[b]	Baseline		End line	
		Time allocation (minutes)	Time x Energy cost[b] (Kcal used)	Time allocation minutes	Time x Energ cost[b](Kcal used)
Personal activities /care					
Sleeping (n=60)	1	600	600x1.0=600.0	515	515x1.0=515.0
Lying, sitting quietly (n=45)	1.2	58	58x1.2=69.6	60	90x1.2=108.0
Eating, drinking (n=60)	1.6	60	60x1.6=96.0	120	120x1.6=192.0
Dressing (n=60)	1.3	35	35x 1.3=45.5	30	35x 1.3=45.5
Shower, Washing (n=60)	1.5	62	62x1.5=93.0	60	60x1.5=90.0
Recreation (n=40)	1.72	130	130x1.72=223.9	60	60x1.72=103.2
Walking, sports (n=60)	3.0	60	60x3.0=180.0	120	120x3.0=360.0
Prayer, moving, strol (n =38)	2.5	30	30x2.5=75.0	90	90x2.5=225.0
Total time used	**1035m ~17.25 h**		**1382.7**	**1035m~17.25 h**	**1638.7**
Household chores					
Washing dishes (n=35)	1.7	30	30x1.7=78.2	30	30x1.7=78.2
House cleaning (n=40)	3.0	55	55x3.0=160.0	55	55x3.0=160.0
Cooking (n=35)	2.0	120	120x2.0=240.0	120	120x2.0=240.0
Washing clothes (n=45)	3.0	20	20x3.0=60.0	20	20x3.0=60.0
Total times		**225 m ~3.75 h**	**538.2**	**225 m ~ .75 h**	**538.2**
Daily trips (n=22)	1.2	60	60x1.2=72.0	60	60x1.2=72.0
Occupational activities n=22)	1.5	125	125x1.5=187.4	123	123x1.5=184.5
Total times		**180 m ~ 3 h**	**259.5**	**180 ~3 h**	**256.5**
Grand total		**1440 m~24 h**	**2180.4±112.4**	**1440 m ~ 24 h**	**2433.4 ±125.4**

[a]PAL = physical activity level, or energy requirement expressed as a multiple of 24-hour BMR
[b]Energy costs (or PAR/physical activity ratio) of activities, expressed as multiples of BMR (basal metabolic rate)

FAO/WHO/UNU, 2001. Human energy requirements, Report of a joint FAO/WHO/UNU expert consultation, Rome 17-24,October 2001

3.9. Analysis the association biochemicals and fasting glucose with sociodemography and nutritional status

In analysis of the association, the influence of sociodemography, nutritional status, and biochemical profileswith or on the plasma glucose level of the zinc supplemented and placebo groups of the studied diabetes subjects was addressed. Assessment was made on the association of the plasma zinc with insulin level, insulin with plasma glucose level, and zinc with stress biomarker malondialdehyde, diet control and physical activity level on the plasma glucose content.

Chi-square analysis indicated that the sociodemography and nutritional status of the studied population did not show any association at the baseline or endline biochemical parameters (table 16,17), except education with HDL (p=0.008). Gender and education showed little had influence (p=0.088, 0.083) on the baseline value. The endline value of biochemical did not indicate any effect on thesociodemography and nutritional status.

Sociodemography and nutritional status indicated had no effect on the plasma fasting glucose level at baseline or endline (table 18).

Table 16: Effect of sociodemography and nutritional status on baseline biochemicals of type 2 diabetes undergoing zinc supplementation

Sociodemography and nutritional status	SGPT (<46 & ≥46)	SGOT (<41 & ≥41)	S creatinine (<1.0 & ≥1.0)	Urine micro albumin (<30 & ≥30)	LDL (<169 & ≥169)	HDL (<37 & ≥37)	Cholesterol (<200 & ≥200)	Triglyceride (<200 & ≥200)
Age in years 35-40 41-50 ≥51	κ^2 =1.182 P=0.124	κ^2 =1.234 P=0.897	κ^2 =0.543 P=0.765	κ^2 =0.745 P=0.507	κ^2 =0.387 P=0.179	κ^2 =0.534 P=0.187	κ^2 =0.987 P=0.510	κ^2 =1.182 P=0.801
Gender Male Female	κ^2 =0.484 P=0.664	κ^2 =1.301 P=0.807	κ^2 =0.629 P=0.233	κ^2 =1.987 P=0.664	κ^2 =1.562 P=0.088	κ^2 =1.892 P=0.515	κ^2 =0.879 P=0.807	κ^2 =2.012 P=0.876
Education Can sign, read, write SSC HSC Graduate and above	κ^2 =2.341 P=0.894	κ^2 =2.452 P=0.275	κ^2 =0.899 P=0.357	κ^2 =0.505 P=0.932	κ^2 =2.503 P=0.083	κ^2 =5.734 P=0.008	κ^2 =1.709 P=0.664	κ^2 =1.386 P=0.762
Occupation Household works Service Others	κ^2 =0.182 P=0.702	κ^2 =0.654 P=0.318	κ^2 =0.456 P=0.131	κ^2 =0.345 P=0.273	κ^2 =0.591 P=0.314	κ^2 =0.649 P=0.930	κ^2 =0.654 P=0.442	κ^2 =0.498 P=0.830
Monthly income 2000-4500 BDT 4501-7000 ≥7001	κ^2 =0.101 P=0.987	κ^2 =0.754 P=0.756	κ^2 =0.562 P=0.751	κ^2 =1.782 P=0.756	κ^2 =0.745 P=0.978	κ^2 =1.345 P=0.585	κ^2 =1.432 P=0.279	κ^2 =1.982 P=0.485
BMI(Kg/m^2) 18.5-24.9 Normal 25-29.9 overweight 30-39.9 Obese Grad I, II)	κ^2 =0.901 P=0.388	κ^2 =1.956 P=0.497	κ^2 =0.512 P=0.732	κ^2 =0.845 P=0.097	κ^2 =0.387 P=0.698	κ^2 =0.546 P=0.562	κ^2 =1.765 P=0.659	κ^2 =1.932 P=0.861

Chi-square test, significance p<0.05

Table 17: Influence of sociodemography and nutritional status on endline biochemicals of type 2 diabetes undergoing zinc supplementation

Socio-demography and Nutritional status	SGPT (<46 & ≥46)	SGOT (<41 & ≥41)	P creatinine (<1.0 & ≥1.0)	Uru-albumin (<30 & ≥30)	LDL (<169 & ≥169)	HDL (<37 & ≥37)	Cholesterol (<200 & ≥200)	Triglyceride (<200 & ≥200)
Age in years 35-40 41-50 ≥51	κ^2=0.900 P=0.962	κ^2=2.221 P=0.654	κ^2=1.762 P=0.229	κ^2=0.364 P=0.406	κ^2=2.98 P=0.243	κ^2=0.712 P=0.156	κ^2=1.629 P=0.935	κ^2=2.854 P=0.579
Gender Male Female	κ^2=2.404 P=0.329	κ^2=1.901 P=0.743	κ^2=0.398 P=0.664	κ^2=1.659 P=0.058	κ^2=1.862 P=0.133	κ^2=0.768 P=0.891	κ^2=0.938 P=0.533	κ^2=1.876 P=0.859
Education Can sign, read and write SSC HSC Graduate and above	κ^2=2.341 P=0.725	κ^2=1.654 P=0.122	κ^2=0.739 P=0.193	κ^2=0.541 P=0.885	κ^2=0.982 P=0.224	κ^2=0.451 P=0.208	κ^2=1.481 P=0.681	κ^2=1.341 P=0.939
Occupation Household works Service Others	κ^2=0.214 P=0.560	κ^2=1.382 P=0.407	κ^2=0.876 P=0.151	κ^2=0.321 P=0.631	κ^2=0.876 P=0.433	κ^2=0.453 P=0.694	κ^2=0.367 P=0.610	κ^2=1.934 P=0.857
Monthly income 2000-4500 BDT 4501-7000 ≥7001	κ^2=1.187 P=0.290	κ^2=1.568 P=0.383	κ^2=0.987 P=0.864	κ^2=0.726 P=0.383	κ^2=0.408 P=0.877	κ^2=0.719 P=0.339	κ^2=0.376 P=0.147	κ^2=1.835 P=0.254
BMI(Kg/m^2) 18.5-24.9 Normal 25-29.9 overweight 30-39.9 Obese grd I, II	κ^2=0.679 P=0.280	κ^2=1.882 P=0.698	κ^2=0.346 P=0.558	κ^2=0.198 P=0.347	κ^2=1.456 P=0.634	κ^2=1.419 P=0.464	κ^2=2.110 P=0.902	κ^2=1.129 P=0.743

Chi-square test, significance p<0.05

Table 18: Influence of sociodemography and nutritional status on fasting blood glucose of Type 2 diabetes undergoing zinc supplementation

Sociodemography and nutritional status	Type 2 diabetes n (%)	Fasting blood glucose µmol/L Baseline ≤11.5 and >11.51	End line <10.0 & ≥10.0
Age in years			
35-40	12 (20.0)	κ^2=1.259	κ^2=0.136
41-50	28 (46.7)	P=0.636	P=1.0
≥51	20 (33.3)		
Gender			
Male	21 (35.0)	κ^2=1.484	κ^2=0.000
Female	39 (65.0)	P=0.223	P=1.0
Education			
Can sign name read, write	19 (31.7)		
SSC	11 (18.3)	κ^2=2.201	κ^2=1.588
HSC	20 (33.3)	P=0.579	P=0.692
Graduate & above	10 (16.7)		
Occupation			
Household works	38 (63.3)	κ^2=1.782	κ^2=0.659
Service	13 (21.7)	P=0.415	P=0.853
Others	09 (15.0)		
Monthly income			
2000-4500 BDT	15 (25.0)	κ^2=2.277	κ^2=1.142
4501-7000	22 (36.7)	P=0.362	P=0.572
≥7001	23 (38.3)		
BMI(Kg/m^2)			
18.5-24.9 (	19 (31.7)		
ormal)	28 (46.7)	κ^2=2.070	κ^2=0.296
25-29.9 (overweight)	13 (21.6)	P=0.376	P=0.880
30-39.9 Obese Grade I, II)			

Chi-square test, significance p<0.05

3.10. Correlation of zinc, insulin, malondialdehyde, fasting glucose, HbA1c

In the zinc supplementation, correlation analysis showed that only plasma zinc and FBG of zinc group had significant (p=0.016) correlation at endline. No significant (P>0.05) difference was observed between plasma zinc and malondialdehyde (MDA) at both baseline and end line. A slightly weak (r=.315) and positive (if zinc increase, MDA will be increased) insignificant relationship observed betweenplasma zinc and MDA at baseline (table 19).However, at the end line this insignificant relation was very weak (-119) andfound to be negative (if zinc increase MDA will be decreased). However, there had a significant (p=0.000) between the baseline and endline value of zinc, insulin, MDA and glucose.

Placebo group did not give any correlation, difference in baseline and endline values was not significant (p>0.05) (table 19).

In logistic analysis (table 21), insulin, fasting glucoe, zinc and MDA were independent variables (regressors) and zinc group and placebo group both are dependent Variables (DVs or regressand). In logistic regression, these 2 dependable variable denoted by binary form, that is, placebo/control=0/ not intervened) and (zinc group/case=1/intervened by Zn). As the baseline results were not significant, it was explained only end line.

There was a18.4 times higher likelihood of having the >17 µmol/L serum zinc in zinc group compared to the placebo at end line. Compared to placebo, zinc group was 6.5 times more likely to have ≤3 mmol /mL serum MDA at end line which indicated lower stressors, might be associated with lower FBS, Higher serum and insulin (whereas they were less likely to have ≤3 mmol /mL MDA at baseline). Having 17.5 times higher level of insulin (>17 µIU/ml compared to ≤17 µIU/ml) in zinc intervention group than placebo at the end line (compared to baseline) could explain positive association of zinc intervention with higher odds of increasing insulin level.

Zinc group was 32.5 times more likely to have less blood sugar (≤10.5 mmol/L) than placebo at end line than baseline.

Table 19: Correlation with plasm zinc, insulin and MDA level in baseline and endline

Correlation	Baseline Mean± SD	Correlation (r)	P-value	Endline Mean± SD	Correlation (r)	P-value	Baseline vs endline
Zinc group							
Zinc and insulin Zinc Insulin	7.9± 4.4 12.0± 2.6	- .226	.115	76.3± 49.3 19.6± 4.7	.159	.270	
Zinc and glucose Zinc FBG	7.9± 4.4 15.5±4.7	-.070	.631	76.3± 49.3 9.4±1.7	.339	.016**	
Insulin and glucose Insulin FBG	12.0± 2.6 15.5±4.7	-.229	.110	19.6±4.7 9.4±1.7	.047	.747	P=0.000
Insulin and MDA Insulin MDA	12.0± 2.6 3.3± 0.9	.034	.816	19.6±4.7 2.6±1.14	.209	.251	
Zinc and MDA zinc MDA	7.9± 4.4 3.3± 0.9	.147	.307	76.3± 49.3 2.6±1.14	-.111	.546	
Placebo group							
Zinc and insulin Zinc Insulin	7.9±5.3 12.1±1.4	.008	.972	9.8±6.2 13.4±2.5	-.217	.358	
Zinc and glucose Zinc FBG	7.9±5.3 12.9±1.6	-.151	.444	9.8±6.2 13.3±2.1	.087	.659	
Insulin and glucose Insulin FBG	12.1±1.4 12.9±1.6	.114	13.4±2.5 13.3±2.1	-.221	.349		P>0.05
Insulin and MDA Insulin MDA	12.1±1.4 3.2±0.9	-.202	.393	13.4±2.5 3.3±0.7	-.147	.587	
Zinc and MDA zinc MDA	7.9±5.3 3.3±0.9	0.315	0.103	9.8±6.2 3.3±0.7	-0.119	0.661	

r= reference category, UOR=Unadjusted odds ratio, 95% CI (L-U) = 95% confidence interval (lower-upper), χ^2= chi-square,Negalkarke R-square=N-R^2, -2 log likelihood Ratio=-2log

Table 20: Association of plasma zinc, Insulin and MDA level with FBS and HbA1c in zinc group

Baseline					End line				
Zn-group Class interval	no	FBS-baseline (≤12 and >12) UOR 95% CI (L-U)	no	HbA1c-endline (≤11% and 11%) UOR 95% CI (L-U)	Zn-group Class interval	no	FBS-endline (≤8 and >8) UOR 95% CI (L-U)	no	HbA1c-endline (≤9% and >9%) UOR 95% CI (L-U)
Insulin (µIU/ml)					Insulin (µIU/ml)				
≤12	35	1.12 (0.354-3.57)	35	1.67 (.563-4.93)	≤19	28	5.0(1.23-0.17)	28	14.14 (1.68-118.4)
>12 (r)	25	Ref category	25	Ref category	>19 (r)	32	Ref category	32	Ref category
		P=.844 -2log=69.53 $N\text{-}R^2$=0.001; X^2=.039		P=.356 -2log=75.53 $N\text{-}R^2$=0.020; X^2=.852			P=0.024 -2log=69.40 $N\text{-}R^2$=0.143; X^2=6.072		P=0.015 -2log=49.81 $N\text{-}R^2$=0.248; X^2=10.237
Zinc (µmol/L)					Zinc (µmol/L)				
≤3	46	0.381 (0.075-1.93)	46	2.538 (.743-8.67)	≤60	20	10.23 (1.23-84.7)	12	1.645 (.392-6.90)
>3 (r)	14	Ref category	14	Ref category	>60 (r)	40	Ref category	48	Ref category
		P=.244 -2log=68.02 $N\text{-}R^2$=.038; X^2=1.572		P=.137 -2log=74.19 $N\text{-}R^2$=.050; X^2=2.197			P=0.031 -2log=59.73 $N\text{-}R^2$=.179; X^2=7.774		P=.496 -2log=59.56 $N\text{-}R^2$=.013 X^2=.487
MDA (mmol /mL)					MDA (mmol /mL)				
≤2 (r)	21	Ref category	21	Ref category	≤2 (r)	34	Ref category	34	Ref category
>2	39	1.16 (0.35-3.81)	39	1.91 (0.62-5.79)	>2	26	17.5 (2.12-144.7)	26	11.9 (1.43-100.0)
		P=.807 -2log=69.53 $N\text{-}R^2$=.001 X^2=.060		P=.254 -2log=75.1 $N\text{-}R^2$=.030 X^2=1.299			P=0.008 -2log=54.54 $N\text{-}R^2$=.287 X^2=12.93		P=0.034 -2log=51.28 $N\text{-}R^2$=.215 X^2=8.765

r= reference category, UOR=Unadjusted odds ratio, 95% CI (L-U) = 95% confidence interval (lower-upper), χ^2= chi-square
χ^2= chi-square,Negalkarke R-square=$N\text{-}R^2$, -2 log likelihood Ratio=-2log

Table 21: Logistic Regression of plasma Insulin, zinc, MDA and FBS on zinc and placebo group

Baseline -Placebo group vs Zn-group			End line - Placebo group vs Zn-group		
	(n)	Odds Ratio 95% CI (Lower-Upper)		(n)	Odds Ratio 95% CI (Lower-Upper)
Insulin (µlU/ml)			**Insulin (µlU/ml)**		
≤12.5 (r)	(45)	Reference category	≤17(r)	(35)	Reference category
>12.5	(25)	1.045 (.355-3.092) P=.937 -2 log=83.75, N-R^2=.000 X^2=.006	>17	(35)	17.471 (3.621-84.29) P=0.000 -2 log=63.824, N-R^2=.355 X^2=19.934
FBS (mmol/L)			**FBS (mmol/L)**		
≤12.5 (r)	(45)	Reference category	≤17(r)	(35)	Reference category
>12.5	(25)	1.045 (.355-3.092) P=.937 -2 log=83.75, N-R^2=.000 X^2=.006	>17	(35)	17.471 (3.621-84.29) P=0.000 -2 log=63.824, N-R^2=.355 X^2=19.934
Serum Zinc (µmol/L)			**Serum Zinc (µmol/L)**		
≤7(r)	(31)	Reference category	≤17 (r)	(34)	Reference category
>7	(47)	0.603 (.229-1.590) P=.307 -2 log=100.773, N-R^2=.019 X^2=1.068	>17	(44)	18.429 (5.717-59.40) P=0.000 -2 log=71.987, N-R^2=.436 X^2=29.854
MDA (mmol /mL)			**MDA (mmol /mL)**		
≤3	(340	Reference category	≤3	(36)	Reference category
>3 (r)	(49)	0.858 (.346-2.128) P=.741 -2 log=108.496, -R^2=.002 X^2=.109	>3 (r)	(21)	6.500 (1.967-21.475) P=0.002 -2 log=67.68, N-R^2=.225 X^2=10.489

3.11. Effect of dietary restriction and physical activity.

Along with zinc supplementation and hypoglycemic therapy, the diabetic patients in the study groups were adviced and attempted to closely monitored to do physical activity or exercise. Both of these activities were observed to assist to some extent lowering the plasma glucose level. Diet restriction reduces and slow the insulin need so that pancreatic β-cells slowly release insulin. And the physical activity increases the muscle oxygen demand, which reduces the oxidative stress of β-cells, and thus stimulates its insulin secretion. Table 23 and 24 show that the diet restriction significantly ($p<0.05$) reduces the calorie intake and physical activity increase the calorie consumption, and makes a significant changes in fasting plasma glucose content.

Table 22. Influence of Dietary Calorie intake and Physical Activity Level on Fasting Blood Glucose of type-2 diabetic patients

Parameter tested				Base line (Mean± SD)	End line (Mean± SD)	Statistics
Energy (Kcal) yielded from daily (24-hours) food intake				3124.7± 453.43	2455.0± 145.36	P=.000*
Fasting plasma glucose (mmol/L)				15.5±4.7	9.4±1.7	P=.000*
Energy (Kcal) used by Different Physical Activities of Daily Life				2180.4±112.4	2433.4±125.4	P=.000*
Fasting plasma glucose/FBS (mmol/L)				15.5±4.7	9.4±1.7	P=.000*
Energy (Kcal) used for 2 different physical activity levels (PALs) by type 2 diabetic patients						
PAL	*Baseline vs. End line (% n)*					
Sedentary activity	68.3 (41)	33.3 (20)	χ^2=4.05	2439.2±131.5	3060.8±390.1	P=.000*
Moderate activity	31.7 (19)	66.7 (40)	P=0.04	2462.5±196.5	3154.4±525.9	P=.000*
Mean energy used for 2 types of PAL				2450.85±164.0	3107.6± 458.0	P=.000*
Corresponding mean FBS (mmol/L)				15.5±4.7	9.4±1.7	

Significance p<0.05

Table 23: Influence of Dietary carbohydrate and Calorie intake on HbA1c% of type-2 diabetic patients

Nutrient consumption	Baseline HbA1c (%) Mean± SD (12.5±2.5) % (n)			End line HbA1c (%) Mean± SD (10.9±1.9) % (n)		
	≤10.5	>10.6	Total	≤10.5	>10.6	Total
Carbohydrate ((g)						
≤300	6.2 (01)	27.3 (12)	21.7 (13)	66.7 (18)	39.4 (13)	51.7 (31)
>300	93.8 (15)	72.7 (32)	78.3 (47)	33.3 (09)	60.6 (20)	48.3 (29)
Total	100 (16)	100 (44)	100 (60)	100 (27)	100 (33)	100 (60)
Statistics	χ^2=1.422	P=0.153		χ^2=4.423	P=0.035*	
Calorie (Kcal)						
≤1900	25.0 (04)	43.2 (19)	38.3 (23)	92.6 (25)	84.8 (28)	88.3 (53)
>1900	75.0 (12)	56.8 (25)	61.7 (37)	7.4 (02)	15.2 (05)	11.7 (7)
Total	100 (16)	100 (44)	100 (60)	100 (27)	100 (33)	100 (60)
Statistics	χ^2=1.614	P=.200		χ^2=.864	P=.442	

Chapter four
Discussion

4. Discussion

Globally diabetes mellitus is a leading cause of death and disability. In Bangladesh, the prevalence of diabetes among adults is increasing substantially; it increased from 5% in 2001 to 9% in 2006 to 2010, and will be 13% by 2030 (Lozano et al, 2012). This pandemic is associated with rapid cultural transforms, growing urbanization, dietary changes, decreased physical activity and other unhealthy lifestyles (Murray et al, 2012). People with diabetes require life-long personal care to decrease the chance of developing long-term complications. Diabetic complications affect the liver, kidney, heart etc vital organs of the body.

The present study was designed to supplement therapeutic zinc to type 2 diabetic patients aiming to reduce plasma glucose level. Zinc tablet (30mg Square Zinc®) was given to the patient daily for three months with monthly follow up and analysis of plasma glucose and insulin. Their sociodemograpic and anthropmetric data and blood specimen were collected. Analysis of plasma HBA1C, zinc, biochemicals, and malonjdialdehyde was made at the baseline (just on reccruitmentof subject) and at the end of 3 month of zinc therapy (endline). Along with zinc intervention, placebo was given to a group of diabetic subjects, which was used as placebo control. Placebo is made of lactose, which is widely used in placebo-controlled clinical study (Atarod et al, 2014) instead of active pharmaceutical ingredient- zinc. The placebo intervention, collection of sociodemographic and anthropometric data and blood specimen, and laboratory analysis of plasma parameters were carried out at same schedule as of the zinc supplemented subjects.In addition, food restriction and physical activity were adviced and controlled. Assessment was made on the sociodemography and nutritional status; biochemicals, zinc, insulin and stress marker malodialdehyde and attempted to evaluate association of these parameters to plasma glucose and glycated hemoglobin (HbA1c). Patient information and analytical data were assessed using SPSS software to interpret and find out results and to make conclusion of the study.

4.1. Study design and population

It was a placebo controlled clinical trial. Clinical trial study is difficult, because it is time consuming and become taxing to the participants. This started recruiting a large number of consentees, but a huge number of them were dropped out during three and half years course of study. Retaining the participants in clinical trial,

particularly with non-hospitalised or out- door patients is troublesome (Fogel et al, 2018; Zweben et al, 2009).

4.2. Socidemography and nutritional status profiles of type-2 diabetic patients

Sociodemographic standing plays a vital role to manage diabetes in the countries whereas, in Bangladesh, an opposite relation was observed between education levels and having diabetes (Lozano et al, 2010). The present study showed that age, education, residential area, nutritional status, and other co-morbid diseases significantly correlated with FBS levels of type 2 diabetes mellitus (T2DM). (There is a significant effect of family history of T2DM on individuals with metabolic syndrome as compared to their counterparts in Asian population. The family history of specific diseases reflects the consequences of genetic susceptibility, shared environment and common behaviors (Murray et al, 2012). The area of residence has an effect on the level of metabolic control, the occurrence of diabetic complications, and quality of life. The countryside people are considered as a low income and education as well as a high number of persons with the disability. In USA, moderately elevated BMI increases the risk of developing T2DM complications. Obesity is a major contributing factor to T2DM and its complications for both men and women (IDF 2012). But the present study results didn't find the association of FBS with sex, BMI, economic condition, and family history of patients with T2DM.

In this study, the socio-demographic and biographic profiles of the patients have been presented in sixty zinc and thirty placebo groups were interviewed and categorized in terms of socioeconomic condition. Assessment of sociodemograpy and nutritional status showed that both of these factors were shown somewhat matched in zinc and placebo group subjects. Diabetic (type 2) was found prevalent among the older aged, married, household working, mid-high income group, and female subjects. This finding was somewhat consistent with report cited (IDF, 2012; Saquib et al, 2010). In the study, socio-demographic and biographic profiles of the patients have been presented in approximately 46% of patients were found within an age range of 35 - ≤50 years. Among the respondent, (41.1%) male and (58.89%) female. Total (98.9%) were married. Most of the patients (34.4%) were educated. Around (42.2%) respondents were from middle-class family. Body Mass Index (BMI) was categorized as normal BMI (28.9%) was less than 25 kg/m2 overweight BMI was (54.4%) between 25 and 29 kg/m2 and as Obese (15.6%) and

morbid obese (1.1%). Patients age in years, zinc group (48.73±7.4) and placebo group (50.27±7.97) respectively. Monthly income zinc group (6220.85±2466.3) and placebo group (6099.6±1634.3). BMI mean zinc group (27.20±3.3) and placebo group (26.9±2.2). Associations between socio demographic factors and FBS levels baseline (14.2±3.9) and end line (9.4±1.7) zinc group was not significant. Placebo group baseline (16.0±3.4) and end line (13.3±2.1) placebo group was not significant.

Aging process develop insulin resistance (Al-Goblan et al, 2014). Diabetic mellitus (T2DM) with obesity and overweight has been predominantly reported elsewhere. Obesity significantly increases the risk of diabetes and high blood pressure, which accounts for 80-85% of the risk of developing type 2 *diabetes*; recently it is suggested that *obese* persons are up to 80 times more likely to develop type 2 *diabetes (www.diabetes.co.uk* › diabetes-and-obesity). In obese individual, cells in fat tissues process more nutrients, which induces inflammation and release cytokines to block the signals of insulin receptors, thus gradually make the cells resistant to insulin.

A very few reports discuss the influence of socio demography or nutritional status on biochemical indices, and some reports regarding biochemical indices of type 2 diabetes undergoing zinc supplementation indicated influence (McClungCai et al, 2014) and others did not find any effect (Oh et al, 2008; Afkhami-Ardekani et al, 2008; Tsutsumi et al, 2003; Farvid et al,2005; Jayawardena et al, 2012; Suliga et al, 2016; Omar A et al, 2018).The present study showed that type diabetic patients were mostly elderly, female, less educated and lower-middle income group of people, which is, to some extent, consistent with findings reported elsewhere (Reza et al, 2018; Islam,2017). Zinc supplementation significantly reduced most of the biochemical indices including the fasting blood glucose. Similar results were also reported elsewhere (Afkhami-Ardekani et al,2008; Partida-Hernandez et al, 2006; Parham et al,2008).

The nutritional status of the zinc supplemented and placebo groups were found to be in good matching. It is required for an experiment case-control trial study (Cologne, Shibata, 1995; RoseLaan et al, 2009; Pearce et al, 2016). It is usually used in case-control study. Matching eliminates confounding factors, most commonly age and sex. It improves the efficiency of the study. In this study, the profiles of sociodemography and nutritional status of the participating diabetics

were well matched, no statistical significance were exist between the zinc supplemented group and the placebo group.

4.3. Change in fasting plasma glucose and glycated haemoglobulin

Zinc supplementation on type 2 diabetic patients to reduce blood glucose level is well documented in previous studies (Saquib et al, 2012). Some investigators have speculated that zinc supplementation could improve glucose tolerance as well as insulin sensitivity in type 2 DM through its zinc effects (Roussel et al, 2003). The potential effects of zinc in diabetes could be related to several mechanisms. Zinc plays a structural role in the maintenance of CuZnSOD structural integrity. Zinc metallothionein complexes in the islet cells provide protection against immune-mediated free-radical attack, and zinc could act also in protecting sulfhydryl groups against oxidation and participate in the inhibition of the free radical production. Hence, zinc could reduce glucose toxicity and contributed in part to the prevention of a decrease of β cell mass and insulin content (Ohlyet et al, 2000). In case of glycated Hb. Zinc therapy also strongly and significantly made a reduction of glycated Hb, but in placebo given group, the glycated Hb was found to increase highly and significantly. It might be because of insulinomimetic potential of zinc (Khanam et al, 2018).

Zinc deficiency reduces insulin secretary reserve and makes glucose intolerance (Kim JLeeet al, 2012), and stimulates glucose oxidation and glycemic control by modulating insulin signaling pathway (Norouzi e t al, 2018). Zinc contribute a vital role in the synthesis, storage and secretion of insulin and its conformational integrity. Zinc deficiency affects the ability of islet cell to produce and secrete insulin (Chausmer et al, 1998). Zinc syntheses proinsulinin pancreatic β-cell, which then makes zinc containing insulin hexamer. Zinc ions also enhance proinsulin'ssolubility and render insulin insoluble-microcrystalline character of the precipitated insulin granule (Omar et al, 2001).

Zinc reduced oxidative stress. Zinc-metaloenzymes such as superoxide dismutase, protect cells and tissues from free radicals insults (Kaur et al, 2014). It acts as a cofactor of the superoxide dismutase enzyme, which regulates detoxification of reactive oxygen species, thus protecting against the oxidative stress induced by chronic hyperglycemia (Cruz et al, 2015).Zinc supplementation

has shown to improve type 2 diabetes symptoms (Begin-Heick et al, 1985). Dietary zincsupplementation has been reported to attenuate hyperglycemia and hyper insulinemia in diabetic patients (Simon et al, 2001). The decreasing HbA1c levels and cholesterol effect by zinc has been reported (El Dib et al, 2015). Zinc supplementation also reported to affect the insulin response differentially, depending on the patient genotype for the SLC30A8 gene encoding the zinc transporter ZnT8 (Maruthur et al, 2015).

Zinc is required for synthesis of insulin hormone, which is arranged in a regular crystalline structure comprising Zn ions in secretory vesicles. Each insulin molecule is linked with 2–4 Zn atoms. A zinc/insulin complex is formed for slow release of insulin into the bloodstream (Prasad et al, 1998). Zinc presents in pancreatic β-cells, and concentrates within the dense core insulin secreting granules (ISG) at around 10 to 20 uM (Hutton et al, 1983). Zn^{2+} ions are an essential for both insulin processing and storage.

Sociodemography and nutritional status did not affect plasma fasting glucose level, but there had a correlation between plasma glucose and zinc supplementation. However, it can be suggested that the lowering of plasma glucose and glycated hemoglobin in the typr 2 diabetes is contributed by insulinomimetic antioxidant zinc therapy.

4.4. Plasma biochemical profiles and association with sociodemography and Nutritional status

Diabetes mellitus is a group of metabolic disorders, in which the most common comorbities involved are impairment of kidney function, ophthalmics, neural function, lipid profiles. It elevates lipids profile (TC, LDL, HDL, TAG), liver function (ALT, AST, ALP, TP, Bilirubin) (Belay et al, 2014), and the kidney function profiles(Adiga, Malawadi, 2016). Fatty liver, osteoporosis and diabetic complications including neuropathy, nephropathy, retinopathy, cardiomyopathy, hypoglycemic coma leadin to death may occur in uncontrolled diabetes (Belay et al, 2014). Further, the diabetes mellitus, insulin and zinc share complex relationship with type 2 DM patients often exhibiting lowered zinc status. Zinc is suspected as having a significant role in normal insulin metabolism. This includes the ability to regulate insulin receptor intracellular events and the ability to support normal pancreatic reaction to a glucose load (Andrews et al, 2005). The type 2 DM patients

are zinc deficient (Farooq et al, 2019; Nadia et al, 2020), which insults the pancreatic β-cells to produce insulin, due to oxidative stress. Zinc supplementation reduces the oxidative stress of β-cells, and thus, actives insulin synthesis, storage, crystallization, and secretion in the pancreatic β-cell, as well as acts on translocation of insulin into the cells (Fernández-Cao et al, 2020).

The main purpose of this study was to evaluate the effect of zinc supplementation on glycemic control in type 2 DM patients. In addition, plasma biochemical profiles of the type 2 DM subjects were analysed and evaluated. In the present study, the biochemicals of lipid profiles, kidney and liver functions were found high, which after zinc therapy, diet restriction and physical activity were significantly reduced. This might be mostly because of antioxidant potential of zinc, which reduces the oxidative stress (Kaur, 201; Cruz et al, 2015), and thus, help normal functioning to lower the liver and kidney function, and the lipid profiles. Similar has also been reported by (McClung,Cai,2014). Attempt had also taken to find out influence of sociodemography and nutritional status on baseline and endline biochemicals of type 2 diabetes. Chi-square test was performed to find the influence or association. However, it did not give any association at the baseline or endline biochemical parameters, except education with HDL. Gender and education showed little had influence on the baseline value. The endline value of biochemicals also did not indicate any effect on the sociodemography and nutritional status.

The socio demography or nutritional status did not find any influence on the biochemical profile, except education on HDL and LDL and gender on urine micro-albumin as well as on the fasting blood glucose value. Some reports also noted the similar results, no beneficial effect (Niewoehner et al, 1986; Seet et al, 2011). This study did find some significant difference in the socio-demographic and biochemical data of type 2 diabetics. It was found that different socio-demographic profiles are significantly correlated with the blood sugar levels of some parameters, which were association with SGPT, SGOT, and Plasma creatinine of T2DM patient. Social demographic factors were associated with several aspects which are patient's age and the male gender can be underlined and family history was not influenced. In biological factors, some association was recorded in socio demography and biochemical profile of have been evidenced in our observation for type 2 diabetes mellitus.

4.5. **Plasma zinc, insulin and malondialdehyde and their association**

Diabetes mellitus, Zinc, insulin, blood glucose as well as stress markers and diabetic mellitus are interrelated and share complex relation. Zinc is suspected to have significant role in normal insulin metabolism. Zinc regulates insulin receptor and supports normal pancreatic reaction to a glucose load (Andrews et al, 2005). Zinc deficiency (Farooq et al, 2019; Nadia et al, 2020) undermines these functions in type 2 DM patients. Supplementation of zinc makes the pancreatic β-cells function.

Zinc supplementation on type 2 diabetic patients to reduce blood glucose level has been well documented in previous studies (Saquib et al, 2012). Some investigators have speculated that zinc supplementation could improve glucose tolerance as well as insulin sensitivity in type 2 DM through its zinc effects (Roussel et al, 2003). The potential effects of zinc in diabetes could be related to several mechanisms. Zinc plays a structural role in the maintenance of CuZnSOD structural integrity. Zinc metallothionein complexes in the islet cells provide protection against immune-mediated free-radical attack, and zinc could act also in protecting sulfhydryl groups against oxidation and participate in the inhibition of the free radical production. Hence, zinc could reduce glucose toxicity and contributed in part to the prevention of a decrease of β cell mass and insulin content (Ohly et al, 2000). Zinc supplementation has been suggested to ameliorate the metabolic disturbance of diabetics (Seet et al, 2011).

The present study showed that zinc supplementation had made a significant rise of plasma zinc (from around 9.0 to 77 µmol/L), insulin level (from 12.0 to 19.5 µIU/ml), that is, from lower to highest level, and had significant reduction in plasma oxidative marker malondialdehyde (from 3.3 to 2.0 1µmol/L). This indicated that the type 2 DM subjects were in severe zinc deficiency, have had lower insulin value and oxidative stress. Zinc supplementation alleviated this disorder. Although, the baseline levels of insulin were within normal range, but at near the lower level. This study showed that plasma insulin and MDA were significantly increased after zinc supplementation in patients with more than 8 years history of diabetics. Previous investigators have hypothesized that zinc enhances tyrosine kinase phosphorylation in the insulin signal transduction from in vitro studies (Simon, Taylor et al, 2000). This study resulted in that the changes of insulin level following

3 months of supplementation reflect an improvement in insulin resistance. Significant changes in fasting blood glucose, insulin and MDA were observed for zinc supplemented diabetics with lower zinc status ($p<0.05$) in this study. A recent randomized, clinical trial reported that 3 months of zinc supplementation (30mg/day) for type 2 diabetics may have beneficial effects in elevating serum zinc level, and improving their glycemic control as shown by decreasing their MDA level (Al-Maroof, Al-Shabatti, 2006).

Few studies on the relationship between oxidative stress and type 2 diabetes have shown that oxidative stress is associated with the development of diabetes complications. Chronic systemic oxidative stress has been shown to cause insulin resistance in rodents (Houstis et al, 2006). The baseline mean serum value of zinc in their study was 68.9±11.9 µg/dL (Kanchana et al, 2005). As compared to the present study, the mean zinc level was higher and mean FBS level was lower. The zinc deficiency in T2D patients may be due to impaired absorption, increased urinary excretion due to altered renal function, or genetic factors or during infections in which zinc has a role. Zinc plays an important role in glycemic control. In physiologic conditions, zinc is abundant in pancreatic islets (Scott et al, 1938; Sondergaard et al, 2006), where it plays a role in the crystallization and secretion of insulin (Li et al, 2014). In addition, evidence suggests that zinc regulates the glucose transporter GLUT4 translocation and the glucose utilization (Tang et al, 2001). Importantly, zinc deficiency is associated with increased chronic inflammation (Wong et al, 2013).

Several mechanisms have been suggested to explain the association between zinc and insulin resistance. Zinc is known to play a major role in the stabilization of insulin hexamers and in the pancreatic storage of insulin because it can enhance insulin binding to hepatocyte membranes (Wijesekara et al, 2009). In fact, reduced hepatic insulin binding to hepatocyte membranes during zinc deficiency may be associated with the contribution of zinc during insulin receptor synthesis (Faure et al, 1992). Furthermore, zinc is an efficient antioxidant, and oxidative stress is considered to be a primary contributor to the initiation and progression of insulin resistance and diabetes (Wiernsperger et al, 2003). In addition, zinc is a component of SOD and is required for optimum SOD activity (Faure et al, 1992). Zinc supplementation at 30 mg daily for 3month improved serum zinc level. In diabetes

plasma zinc concentration should be kept within the normal range, otherwise insulin-resistance as well as diabetes complications will be worsening. In case of hypozincemia zinc supplementation improves glycemic control. Collectively, if diabetic patient had low zinc concentration, permanent zinc supplementation is needed and should be administered in supra physiological doses. Contemporary formulations, especially nano-formulations are effective in normalization of zinc homeostasis and deregulations of diabetes originate in hypozincemia can be corrected by zinc supplementation.

Zinc supplementation for type-2 diabetics has beneficial effects in elevating their serum zinc level, and in improving their glycemic control that is shown by decreasing their HbA1c% concentration. These data demonstrated potential beneficial antioxidant effects of zinc supplementation in persons with type 2 DM. The mechanism of this action could be due to the antioxidant effects of zinc, especially in protecting SH groups, but also the modulating effect of zinc on insulin sensitivity cannot be ruled out. These results are particularly important in light of the deleterious consequences of oxidative stress in persons with diabetes. Increased intake of zinc, in addition to other nutritional and pharmacological treatments, may be important in the delay and/or prevention of the complications of diabetes.

It has also been reported that zinc has immune enhancing (McClung, Cai, 2014). Zinc contributes vital function in immunity, particularly, important in cell-mediated immunity (Mooradian et al, 1988; Eliashiv et al, 1978). Zinc therapy enhances immunity through increasing of CD4 T–cells. Zinc controls insulin signaling, glucose use, maintain lipid metabolism and cell functions. Zinc deficiency induces chronic disease including diabetes, which itself also makes zinc deficiency. Zinc therapy can prevents development and or the progression of diabetic nephropathy.

Further, Zinc has anti–inflammatory property (Mooradian et al, 1988: Khan et al, 2013). Many investigations reported function of zinc in the regulation of metabolic syndrome. It controls cytokine expression, and inflammation suppresses suppresses (Olechnowicz et al, 2018). Inflammation associated with insulin resistance and diabetes (Dandona et al, 2004; Grimble et al, 2002). Zinc deficiency occurs in inflammation and type 2 diabetes (Giacconi et al, 2005), which is due to high IL-6 production that associates with insulin resistance, hyperglycaemia and dyslipidemia. The -209 A/G MT2A polymorphism is involved in chronic

inflammation (high plasma IL-6), hyperglycaemia, enhanced HbA1c, which are manifested by marked zinc deficiency. Therefore, zinc therapy may overcome the diabetic inflammation.

Supplementation of zinc to type II DM patients demonstrated better glycemic control and desirable changes in lipid profile, MDA level, Insulin level, liver function, as well as improvement in kidney functions, therefore, zinc may have supplementary benefits in the routine management of adult DM and could be a possible strategy favoring the life quality of those who have risk factors for other diseases in addition to diabetes. Zinc supplementation at 30 mg daily for 12 weeks beneficially increased serum zinc by 70%, insulin level increased and decreased stress marker level.

The results of the current study showed that zinc supplementation in a dose of 30 mg/day orally for 3 months significantly decreases fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) in type 2 DM patients. These results are in agreement with previously published data that showed improvement in glycemic control with zinc supplementation (Hussainet al, 2006; Gunasekara etal, 2011). Meanwhile, Oh and Yoon, 2008 analyzed the effect of zinc supplementation on glycemic control by the baseline HbA1c level of diabetes subjects (Baseline 13.4±3.9 and end line 10.9±1.9), in comparison this study found significant decrease in HbA1c after 3 months of supplementation. In case of glycated Hb. However, zinc therapy also significantly made a reduction of glycated Hb, which might be because of insulinomimetic potential of zinc (Khanam et al, 2018).

In summary, this study indicated that significant improvement of fasting insulin level as well as decreased MDA level were observed in zinc supplemented diabetic patients with shorter diabetic duration, poorer glycemic control, and marginal zinc status. However, further investigation is needed before a firm conclusion could be drawn for the relationship between zinc supplementation and glycemic control.

4.6. Diet restriction and blood glucose of type 2 diabetes

Zinc is one of the most important essential trace elements in human nutrition and lifestyle. Its deficiency may severely affect the homeostasis of a biological system. This experimentally-controlled nutritional study provides clear evidence of beneficial impacts of zinc supplementation on glycemic control and pancreatic islets

regeneration in diabetic patients, which could be attributed to its ant diabetic and antioxidant properties. Therefore, consumption of zinc-rich diets or otherwise, zinc supplements, should be encouraged with moderation in diabetics for optimal glycemic control.

The magnitude of Diabetes mellitus (DM) has increased dramatically in many parts of the world and this chronic metabolic disorder is one of the non-communicable disease and the biggest global public health problems in the past 20 years (Gelaw et al, 2018).) Just under half a billion people were lived with diabetes worldwide in 2019 (9.3% prevalence) and the number is projected to increase by 25% (578 million) in 2030 and 51% (700 million) by 2045 with linked health, social, and economic costs. Diabetes is associated with rapid cultural transforms, growing urbanization, dietary changes, decreased physical activity and stress induced digital unhealthy lifestyle and are responsible for the large volume of T2DM patients worldwide (Fareed et al, 2017; Asif et al, 2014; Danaei et al, 2011). As DM is characterized by chronic increase in the blood-glucose level resulting from a relative insulin deficiency or insulin resistance or both includes chronic hyperglycemia with disturbances of carbohydrates, proteins and fat metabolism that affect the vital organs liver, kidney and heart of the body. The etiology of T2DM is complex and is associated with irreversible risk factors such as age, genetic, race, and ethnicity and reversible factors such as diet, physical activity and smoking (Gelaw et al, 2018; Sami et al, 2017).

Dietary factors are important in the management and prevention of type 2 diabetes (Farouche et al, 2010).Antioxidant rich (green-yellow fruits and vegetables), carbohydrate restricted low GI (glycemic index), and high fiber containing complex carbohydrate (whole grains) diets have been suggested to reduce or slow insulin need, protect against oxidative stress, thus, maintaining glucose homeostasis (Russell et al, 2017). Diet modification (Forouhi et al, 2018; Meng et al, 2017; Aggarwala et al, 2016) and physical activity transforming from sedentary lifestyle to moderately active life are essential for efficacy of hypoglycemic agents (Asif et al, 2014; Russell et al, 2017). Balanced diet given in divided time interval can work in prevention and reverse of diabetes (Asif et al, 2014).

In this study diet counseling showed a significant reduction of glycosylated hemoglobin (HbA1c) and fasting blood glucose (FBS) level among the uncontrolled

type 2 diabetic patients (T2DM). In this study, rice intake was reduced to 100 g from baseline intake of 277 g after 3 months of dietary intervention. A study (Reza et al, 2018) mentioned 92% carbohydrate intake (mainly rice) by diabetic patients and showed significant association between rice intake and an increased risk of type 2 diabetes in women (Nanria et al, 2010). This study showed a contribution of total carbohydrates to the total dietary intake of T2DM patients was almost 50% in baseline reduced to 40.6% in end line. It was found that 10% reduction of dietary carbohydrate from baseline contributed to the subsequent reduction of calories (from 3124.7 to 2455 kcal), which had greater influence on reducing fasting blood glucose (baseline: 15.5 to end line: 9.4 µmol/L). A recent study (Shehab et al 2011; Nghia et al, 2019) showed more carbohydrate contribution (≥60.0%) to diet and its association with fasting blood sugar like this study. Moreover, lower proportion of carbohydrate intake at the end o 3 months zinc therapy was found tobe associated with lower HbA1c% among T2DM patients. Recent analysis (Meng, Bai, 2017) also found that low carbohydrate diet (LCD) had significant effect on HbA1c level.

A high fat content in the diet may result in deterioration of glucose tolerance. It is by many mechanisms including decreased binding of insulin to its receptors, impaired glucose transport, and reduced proportion of glycogen synthase and accumulation of stored triglycerides in skeletal muscle (Pan et al, 1997; Grundleger et al, 1982). The fatty acid composition of the diet, in turn, affects tissue phospholipid composition, which may relate to insulin action by altering membrane fluidity and insulin signaling (Storlien et al, 1996). High fat intake has also been associated with higher fasting insulin concentrations (Marshall et al, 1997), and a lower insulin sensitivity index(Lovejoy et al, 1992). Some studies did not give any association between diabetes risk and total fat intake (Salmeron et al, 1997; Meyer et al, 2001).

Few human intervention studies have examined the effects of high fat, low carbohydrate diets on diabetes risk, and the results reported inconsistent (Kolterman et al, 1979; Lovejoy et al, 1998; Bisschop et al, 2001). Higher proportions of saturated fatty acids in serum lipids/muscle phospholipids have been associated with higher fasting insulin levels (Folsom AR et al, 1996), lower insulin sensitivity (Vessby B et al, 1994) and higher risk of developing type 2 diabetes. Higher vegetable fat (unsaturated fat) and intake of PUFA have, in turn, been associated with a lower risk of type 2 diabetes as well as lower fasting and 2-hr

glucose concentrations. In two short-term studies with single saturated fatty acids—lauric, palmitic and stearic acids reported no effect on glucose and insulin metabolism (Schwab et al, 1995).

In the light of present knowledge regarding the relationships between type 2 diabetes and dietary fat was scared. Most intervention studies aimed at investigating the effect of fish oil on insulin sensitivity have been done in patients with type 2 diabetes and was negative (Borkman M et al, 1989; Annuzzi G et al, 1991). There have marked differences in various countries of the world with respect to the fat-to-carbohydrate ratios consumed by different populations. A significant positive association has been shown between dietary fat consumption and the proportion of the population who are overweight (Richards et al, 2001).High carbohydrate intake decreases the prevalence of diabetes(Marshall et al, 1991; Tsunehara et al, 1990). However, many studies have reported that an increased intake of carbohydrates can reduce HDL levels and raise fasting plasma triacylglycerol oncentration (Parks et al, 2000). It is known that a high carbohydrate intake increases the requirement for insulin secretion in order to maintain glucose homeostasis. Insulin secretion by beta-cells is glucose sensitive and a high intake of carbohydrate in relation to energy intake produces higher post-prandial insulin levels. It is possible that repeated stimulation of high insulin output by a high carbohydrate diet could speed up an age-related decline in insulin secretion leading to an earlier onset of type 2 diabetes (Rasmussen et al., 1993). The quality and quantity of carbohydrate may speed up this response. The FAO/WHO recommended55 % carbohydrate in total energy intake.

This study showed that the influence of restricted dietary intake on both the blood glucose and glycated hemoglobin level (HbA1c %) of type 2 diabetic patients. Dietary counseling given to them focused mainly on simple carbohydrate restriction in diet (more complex carbohydrate e.g. vegetables, fruits and whole grains enhancement), At the end of counseling, HbA1C, FBS, 24-hour dietary intake were followed up, collected and recorded in the same structured questionnaire after 3 months. It was indicated that carbohydrate restricted diet apparently reduced the blood glucose level and HbA1C of the type 2 diabetic patients.

4.7. The essential role of Physical activities management of type 2 diabetics

In this study, counseling on doing physical activity, especially walking, showed significant reduction of glycosylated hemoglobin (HbA1c) and fasting blood glucose (FBS) level among the type 2 diabetic patients. A single short time physical exercise (brisk walking) has significant insulin like effect on fasting and random blood sugarand HbA1c.

Studies have shown that physical activity plays an important role on glycemic control (Balaji et al, 2017; Aggarwala et al, 2016; Shehab et al., 2011). Even brisk walkinglowers blood glucose and combined resistance had greater impact on glycemic control than both alone. Study reported small meals with more meal frequency are helpful in lowering blood sugar (Asif et al, 2014). In this study replacing some sedentary activities with physical activities like walking and sporting reported significant increase of energy expenditure, which subsequently decreased fasting blood glucose.

Immobility or Inactivity is one of the causes of insulin resistance which can direct to T2D.Exercise can be used as a treatment, which can affect glucose transpoter-4 (GLUT 4) translocation, and can improve insulin signaling (Kraniou et al, 2006).Regular physical activity can alsolikely effect hypoglycemic agents. In addition, exercise canstep up glucose tolerance. Exercise has also been reported to improve pancreatic β cell function in obese diabetics(Pedersen et al, 2009).

This study recruited 90 participants who were randomly assigned to either a diabetic group. The diabetic group initially took physical activities daily. The lifestyle intervention group was told to lose approximately 7% of their body mass through a healthy low fat, low calorie, and to engage in physical activity for at least 150 minutes per week. Both HbA1c as well as diagnosis of T2D were tracked over four years at 3months intervals. Using exercise as a treatment can be an effective way to improve clinical markers of diabetes such as HbA1c and fasting glucose levels, and more importantly exercise can help control post-prandial glycemic excursions (Barr et al, 2002; Larsen et al, 1997). These three parameters are used to diagnose and monitor T2D. These parameters can be affected by exercise by reducing the amplitude of a post-prandial glycemic excursion. Studies have shown that exercise or lifestyle modification can be as effective if not more so than pharmacological interventions at preventing and treating T2D.

The results of the study showed, as expected, that the placebo group had the highest incidence of diagnosis with T2D. By 3 months into the study, there was statistically significantly higher incidence of T2D in the placebo group compared with zinc group, and this significance would persist until the end of the study. Thus, lifestyle was the most effective at preventing T2D in a at risk population. There were also differences seen in HbA1c and fasting plasma glucose levels between the groups. As expected, the placebo group had not statistically significantly higher fasting plasma glucose levels as well as HbA1c throughout the study. The zinc group and lifestyle group both had similar fasting plasma glucose levels throughout the study, their HbA1c differed. The zinc group had statistically significantly lower HbA1c compared with the placebo group between 3 months of the study.

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Therapeutic use of zinc in lowering of blood glucose in Type 2 Diabetic Patients: A case controlled clinical study (manuscript submitted)

Stress relieving potential of therapeutic zinc in Type 2 Diabetic Patients

প্রশ্নাবলী

Dietary habit and sitting time of NAFLD patients in Bangladesh

সাক্ষাতকারের তারিখ : ক্রমিক নং .....

স্থান : .....

পরিবার পরিচিতি, গঠন ও আর্থসামাজিক তথ্য

১. আপনি কোন ধর্মাবলম্বি? (কোড: ১=মুসলিম, ২=হিন্দু, ৩=বৌদ্ধ, ৪=খৃষ্টান) ☐

পরিবারের সদস্য সংখ্যা- ☐☐

৩. জরিপ এলাকা : (১= শহর এলকা, ২= উপজেলা, ৩= গ্রাম) ☐

ক. পরিবারের সদস্যদের পরিচিতি ☐

MID	পরিবারের সদস্যদের নাম	লিঙ্গ কোড-১	পরিবার প্রধানের সাথে সম্পর্ক কোড-৩	ডায়াবেটিক আবস্থা		বয়স		বৈবাহিক অবস্থা কোড-২	পাশকৃত শ্রেণী শিক্ষা কোড-৬	পেশা কোড-৫		ওজন	উচ্চতা	মধ্যমা হ	এনএফএলডি কোড-৭	
				হাঁ-১,	না-২	বছর	মাস			প্রথম	২য়				এনএফএলডি কোড-৭	বছর
1.																
2.																
3.																
4.																
5.																
6.																
7.																
8.																
9.																
10.																

কোড-১: লিঙ্গ
১= পুরুষ
২= মহিলা
৩= গর্ভবতী
.
৫= গর্ভবতী ও স্তন্যদায়ী

কোড-২: বৈবাহিক অবস্থা
১= অবিবাহিত
.
৫= তালাকা প্রাপ্ত/প্রাপ্তা.

কোড-৩: পরিবার প্রধানের সাথে সম্পর্ক
১= পরিবার প্রধান
২= স্ত্রী/স্বামী
.
১৩= স্থায়ী ভৃত্য

কোড-৫: পেশা কোড
১= কৃষি (ফসল)
২= মাটি কা
.
.
১৯= অন্যান্য

কোড-৬: শিক্ষা কোড
১= নিরক্ষর
.
৯= স্নাতক
১১= অন্যান্য --------

কোড-৬: পরিবারের ডায়াবেটিস
১= বাবা/মা
.
.
৬=নানা/নানী

লক্ষন ছিল
১= ঘন ঘন প্রস্রাব
.
৯= পাকস্থলি প্রদাহ

জটিলতায়
১= উচ্চ রক্তচাপ
.
.
১৩= দ্রুত শ্বাসপ্রশ্বাস
১৫= দৃষ্টিশক্তি কমা

পরিবারের মাসিক আয় ব্যয় সংক্রান্ত তথ্য:

৪। পরিবারের মাসিক মোট আয় কত? ..... টাকা

৫। পরিবারের মাসিক ব্যয় (নিম্নের খাত ওয়ারী)

কোড	ব্যয়ের খাত	টাকার পরিমান	কোড	ব্যয়ের খাত	টাকার পরিমান	কোড	ব্যয়ের খাত	টাকার পরিমান
১	খাওয়া		৪	ডায়াবেটিস চিকিৎসা		৭	বসবাস	
২	শিক্ষা		৫	যাতায়াত		৮	কৃষি	
৩	ঔষধ		৬	পোষাক-পরিচ্ছেদ		৯	অন্যান্য	

১১। **আপনি কোন ডায়াবেটিক সেন্টারের সাথে জড়িত আছেন কি?** (১ =হাঁ, ২ = না) ☐

১২। **আপনি কোথায় ডায়াবেটিক পরীক্ষা করান–** (১ =বাসায় নিজে, ২ =ডায়াবেটিক সেন্টার, ৩ =ফার্মেসী, ৪ = কোন ডায়াগনষ্টিক সেন্টার) ☐

১৩। **আপনি কি ডায়াবেটিক ঔষধ গ্রহণ করেন?** (১ = হাঁ, ২ = না) ☐
যদি হাঁ হয় তা হলে ঔষুধের নাম উলেখ করন .....

১৪। **আপনি কত ইউনিট ইনসুলিন গ্রহণ করেন** (১ = হাঁ, ২ = না) ☐
যদি হাঁ হয় তা হলে কত ইউনিট উলেখ করন .....

১৫। **স্বাক্ষাতের তারিখ/দিন থেকে গত তিনমাস কি কি ধরনের অসুখে ভূগেছেন?**

রোগের নাম (কোড)	কত দিন ভুগেছেন	কোথায় চিকিৎসা নিয়েছেন	কত টাকা ব্যয় হয়েছে	কতদিন হয় সুস্থ্য হয়েছেন
১. হার্টের রোগ ২. ষ্ট্রোক ৩. চোখের রোগ/অন্ধত্ব ৪. কিডনি রোগ ৫. পায়ে ঘা/পা কেটে ফেলা ৬. লিভারের রোগ				

১৬। **গত তিনমাস কতবার চোখের পরীক্ষা করিয়েছেন?** ☐

১৭। **গত তিনমাসে কতবার পাপরীক্ষা করিয়েছেন?** ☐

১৮। **আপনি কি উচ্চ রক্তচাপের ঔষধ গ্রহণ করছেন?** (১ = হাঁ, ২ = না) ☐
করে থাকলে কি ঔষধ সেবন করেছেন? উলে- খ কর ন .....

১৯। **আপনি গত তিনমাস কতবার কিডনি পরীক্ষা করিয়েছেন?** ☐

(গ) **ডায়াবেটিসে আক্রান্তদের খাদ্যাভাসের তথ্য:**

২০। **আপনি ধুমপান /তামাক গ্রহণ করেন কি?**(1 = হাঁ, 2 = না) ☐

২১। **আপনি পান খান কি?** (1 = হাঁ, 2 = না) ☐

২২। **আপনি কি চিনির তৈরী মিষ্টি জাতীয় খাদ্য গ্রহণ করেন কি?** (1 = হাঁ, 2 = না) ☐
যদি হাঁ হয়, মাসে কতবার .....

আপনি নিম্নলিখিত খাবারগুলো গত ৭দিনে কতবার গ্রহণ করেছেন (Recall Method):

খাবারের মেনু	সকালের নাস্তা	মধ্যবর্তী সময়ে গৃহীত খাবার	দুপুরের খাবার	বিকেলের নাস্তা	রাতের খাবার
শর্করা জাতীয় খাদ্য (সিদ্ধ চাল, আতপ চাল, আটার রুটি, পাউরুটি, ব্রেড রোল, নানরুটি, ভাত, মুড়ি, চিড়া, খৈ, নুডুলস, সুজি, সেমাই, বার্লি, সাগু, আলু, মিষ্টি আলু, নোনতা বিস্কুট, পাকা কলা,পরিজ, ভুট্টা, আটা-ময়দা-চালের গুড়া)					
প্রোটিন জাতীয় (মাছ, মাংস, মুরগীর মাংস, কলিজা, ছানা, পনির, চিনাবাদাম, ডিম)					
দুধ ও দুগ্ধজাত খাদ্য(তরল দুধ, গুড়ো দুধ, গুড়ো দুধ, দই, ছানা, পনির)					
ডাল জাতীয় খাদ্য (ডাল, ছোলা ভাজা, সয়াবিন, সিম বিচি, মটর শুটি, কাবলী মটর)					
ফ্যাট ও তেল জাতীয়(তেল সব রকম, ঘি, ডালডা, মাখন)					
শাক ও সবজি (গ্রহনযোগ্য)ঃ পালং শাক, লাল শাক, পুঁই শাক, কলমি শাক, ডাটা শাক, কচু শাক					
সবজি (অগ্রহনযোগ্য)ঃ আলু, মিষ্টি কুমড়া, কচু, থোর, বিট, কাঁচা কলা, বরবটি, মোচা, সিম, গাজর, কাঁকরোল, সিমের বিচি, কাঁঠালের বিচি, শালগম, ইঁচর, ঢেঁড়স, বেগুন, মটর শুটি					
ফল (গ্রহনযোগ্য)ঃ কাল জাম, লেবু, আমড়া, জাম্বুরা, কামরাঙ্গা, বাঙ্গি, জামরুল, আমলকি, কচিডাবের পানি					
ফল(অগ্রহনযোগ্য)ঃ আম, পাকা পেয়ারা, লিচু, আতা ফল, কাঁঠাল, কমলা, আপেল, মাল্টা, পাকা পেপে, পাকা কলা, নারিকেল, মিষ্টি বরই, তরমুজ, বেদানা, পাকা বেল, নাসপাতি, আঙ্গুর, আনারস, কেশর, তাল					

২৩। **আপনি দিনে কতবার খাদ্য গ্রহণ করেন?** (1= ১ বার, 2= ২ বার, 3= ৩ বার, 4= ৪ বার, 5= ৪এর অধিক) ☐

২৪। **আপনি কি নন ডায়াবেটিসের সাথে খাদ্য গ্রহন করেন?** (1 = হাঁ; 2 = না) ☐

২৫। **আপনার খাবার আপনি নিজে পছন্দ করেন নাকি পথ্য নির্দেশিকা মেনে চলেন?** (1 = হাঁ; 2 =না) ☐

২৬। প্রতিদিন কি ধরনের খাদ্য গ্রহণ করেন? ☐☐
1 = উচ্চ ক্যালরী সম্পন্ন, 2 = মধ্য ক্যালরী সম্পন্ন, 3 =নিম্ন ক্যালরী সম্পন

২৭। **মিষ্টি জাতীয় খাবার গ্রহণ করেন কি?** যদি হাঁ হয় ☐
1 = প্রতিদিন, 2 = সাপ্তাহিক, 3 = মাসিক, 4 = ত্রৈমাসিক, 5 = ষান্মাসিক, 6 = বাৎসরিক

পরিস্কার পরিচ্ছন্নতা

২৮। বাসস্থানের অভ্যন্তরে পরিস্কার পরিচ্ছন্নতা কি ধরণের? (১= পরিস্কার পরিচ্ছন্ন, ২= অপরিস্কার, ৩=স্যাঁতসেতে, ৪= পরিস্কার কিন্তু স্যাঁতসেতে ☐

২৯। পরিবারের পাঁচ বছরের কম বয়সী শিশুরা কোথায় মলত্যাগ করে? (১= পায়খান, ২ = খোলা/ঝুলন্ত পায়খানায়, ৩= নির্দিষ্ট গর্ত/ভুয়া/চাড়ি, ৪= স্বাস্থ্য সম্মত (স্যানিটারী/স্লাব) পায়খানায়, ৫= খোলা জায়গায়, ৬=ড্রেনের ধারে, ৭=প্রযোজ্য নয়, ৮ = অন্যান্য ---------- ☐

৩০। পায়খানা বা নোংরা জায়গায় যাওয়ার সময় জুতা/স্যান্ডেল ব্যবহার করেন কি? (১= হাঁ; ২= না) ☐

তথ্য সচেতনতা

৩১। আপনি কি খবরের কাগজ পড়েন? (১= হাঁ, ২ = না; ৪= প্রযোজ্য নয়) ☐

৩২। আপনি কি খবরের কাগজ রাখেন এবং পরিবারের সদস্যরা পড়ে? (১= রাখি এবং সদস্যরা পড়ে. ২= হাঁ রাখি কিন্তু বেশীর ভাগ সদস্য পড়ে না, ৩= রাখিনা, এখানে খবরের কাগজ পাওয়া যায় না, ৫= খবরের কাগজের অনেক দাম এজন্য রাখি না; ৬= প্রযোজ্য নয়) ☐

৩৩। আপনি কি রেডিও শুনেন? (১= হাঁ, ২= না, ৩ = প্রত্যেক দিন শুনি, ৪= সপ্তাহে অন্তত একদিন শুনি, ৫= সপ্তাহে একদিনের বেশী শুনি; ৬= প্রযোজ্য নয়)

৩৪। আপনারা কি টেলিভিশন দেখেন? (১=হাঁ, ২= না, ৩= প্রত্যেক দিন দেখি, ৪= সপ্তাহে অন্তত একদিন দেখি, ৫ = মাঝে মধ্যে দেখি; ৬=সপ্তাহে একদিনের বেশী দেখি)

৩৫। তথ্য আদান প্রদানে কোন মাধ্যমটি আপনি বেশী ব্যবহার করেন? (১= ল্যান্ড ফোন, ২= মোবাইল ফোন, ৩= চিঠি, ৪= কোনটাই নয়, ৫ = অন্যান্য .....)

গৃহ নির্যাতন (সকল পরিবারের জন্য প্রযোজ্য)

৩৬। স্ত্রীর কর্মকান্ডে অনেক সময় স্বামীরা রাগান্বিত হয় ও স্বামী স্ত্রীকে মারে, ইহা কি যৌক্তিক বলে মনে করেন? (১= হাঁ, ২ = না)

৩৭। নিম্নলিখিত কারণ গুলোতে স্বামী স্ত্রীকে প্রহার করা উচিৎ বলে আপনি মনে করেন কি?

ক) স্বামী কে না জানিয়ে বাহিরে যাওয়া? (১= হাঁ, ২ = না)

খ) সন্তানদের সঠিক ভাবে যত্ন না করা? (১= হাঁ, ২ = না)

গ) স্বামীর সাথে কোন বিষয়ে তর্ক করা। (১= হাঁ, ২ = না)

ঘ) সহবাসে অনিহা প্রকাশ করে। (১= হাঁ, ২ = না)

ঙ) বাড়ীর বড়দের সম্মান না করা বা কথা না শুনা? (১= হাঁ, ২ = না)

৩৮। আপনার বাবা আপনার মা কে মেরেছে এমনটি কখনও শুনেছেন কি? (১= হাঁ, ২ = না)

৩৯। আপনার বাবা আপনার মা কে মেরেছে, এমনটি দেখেছেন কি? (১= হাঁ, ২ = না)

পুষ্টিজ্ঞানের অনুশীলন

৪০। আপনি আপনার সন্তানকে জন্মের পর বুকের দুধ খাইয়েছিন কি? (১= হাঁ, ২= না, ৩= প্রযোজ্য নয়)

৪১। আপনার সন্তানকে জন্মের প্রথম তিনদিন পর বুকের দুধ ছাড়া অন্য কোন তরল খাদ্য দিয়েছেন কি? (১= হাঁ, ২= না, ৩= মাঝে মধ্যে, ৪ = প্রযোজ্য নয়, ৫ = অন্যান্য .....)

৪২। আপনি সুষম খাদ্য খান কি? (১= হাঁ; ২ = না; ৩= প্রযোজ্য নয়)

৪৩। শরীরে কখনও রক্তের পরিমাণ না কমার জন্য প্রতিদিন নিয়মিত আপনি কি কি খাবার খান? (১ = বেশী দামী খাবর; ২= শাক-সবজি, ফলমুল; ৩= ভাত, মাচ, ডিম, দুধ; ৪=কোন কিছুই খাইনা; ৫=জানা নাই; ৬= প্রযোজ্য নয়)

৪৪। আলু, মাছ/মাংস, ডালও শাকসবজি এই খাবারগুলোর মধ্যে আপনি আয়রন সমৃদ্ধ খাবার হিসাবে কোনটি খান/খেয়েছেন? (১= আলু; ২=মাছ/মাংস; ৩=ডাল; ৪=শাক-সবজি; ৫= প্রযোজ্য নয়)

৪৫। আপনি যখন গর্ভবতী ছিলেন তখন কি নৈমিক অন্যান্য খাবারের পাশাপাশি বেশী বেশী শাক-সবজি, ফল-মুল খেয়েছেন /খেয়েছিলেন? (১= হাঁ; ২=না; ৩=মাঝে মধ্যে খেয়েছি; ৪=প্রযোজ্য নয়)

রান্না ও পরিস্কার-পরিচ্ছন্নতা সম্পর্কিত অনুশীলন:

৪৬। আপনারা রান্নার পূর্বে হাত পরিস্কার করে নেন কি? (১= হাঁ; ২= না; ৩= কখনও কখনও; ৪= প্রযোজ্য নয়)

৪৭। ভাত রান্নার সময় ভাতের মাড় ফেলে দেন কি? (১= হাঁ; ২= না; ৩= মাঝে মধ্যে ফেলে দেই; ৪= প্রযোজ্য নয়)

৪৮। রান্নার সময় শাকসবজি সিদ্ধ করার পর পানি ফেলে দেন কি? (১= হাঁ; ২=না; ৩= প্রযোজ্য নয়)

৪৯। রান্নার সময় কি ধরনের লবন ব্যবহার করেন? (১=প্যাকেট/আয়োডাইজড লবন; ২= খোলা লবন; ৩= প্রযোজ্য নয়)

৫০। আপনারা খোলা এবং বাসি খাবার খান কি? (১=হাঁ; ২=না; ৩= কখনও কখনও; ৪= প্রযোজ্য নয়)

পানির উৎস ও পানি বিশুদ্ধ করণ

৫১। আপনাদের খাওয়ার পানির প্রধান উৎস কি? (১= নলকূপ/ট্যাপ; ২= কূয়া; ৩= পুকুর/ডোবা; ৪=নদী/খাল; ৫=ঝর্ণার পানি; ৬= বোতলের পানি; ৭=পুকুর/নদীর পানি ফুটিয়ে; ৮= প্রযোজ্য নয়)

৫২। রান্না ও ধোয়া-পাখলায় (থালা, বাসন, হাড়ি, পাতিল) পানির উৎস কি? (১=নলকূপ/ট্যাপ; ২=কূয়া; ৩=পুকুর/ডোবা; ৪= নদী/খাল; ৫= প্রযোজ্য নয়)

৫৩। পানি পান করার পূর্বে পানি কিভাবে বিশুদ্ধ করেন? (১= কতক্ষন ফুটান ..... মিনিট; ২= পানি বিশুদ্ধ করন ট্যাবলেট দিয়ে; ৩= ফিটকারী দিয়ে; ৪= ফিল্টার; ৫= বিশুদ্ধ করা হয় না; ৬= টেপ/টিউবওয়েলের পানি তাই বিশুদ্ধ করা হয় না?; ৭= প্রযোজ্য নয়)

৫৪। টিউবওয়েলের পানি পান করলে সে পানি আর্সেনিক মুক্ত কি? (১= হাঁ; ২ = না; ৩= এখনো নির্ধারণ করা হয় নি; ৪= প্রযোজ নয়)

পরিবারের পয়ঃনিস্কাশন

৫৫। বাড়ির ময়লা আবর্জনা কোথায় ফেলেন? (১= নির্দিষ্ট গর্তে/জায়গায়; ২ = বাড়ির আশেপাশে খোলা জায়গায়; ৩= যেখানে সেখানে; ৪= প্রযোজ্য নয়)

৫৬। পরিবারের কিশোরী/মহিলারা কোথায় গোসল করেন? (১= পুকুর; ২= খাল/নদী; ৩= গোসলখানা/ নলকূপ; ৪= ডোবা; ৫= প্রযোজ্য নয়) ☐

৫৭। পরিবারের সদস্যগণ কি ধরণের পায়খানা ব্যবহার করেন? (১= পায়খানা নাই; ২= খোলা/ঝুলন্ত পায়খানা; ৩= নির্দিষ্ট গর্ত/কুয়া/চাড়ি; ৪= স্বাস্থ্য সম্মত (স্যানিটারী/স্লাব) পায়খানা; ৫ = প্রযোজ্য নয়) ☐

৫৮। শিশু থেকে কিশোর কিশোরী পর্যন্ত বয়সের ডায়াবেটিক রোগী হলে তার তথ্য:

(ক) আপনার সর্বশেষ সন্তান জন্মের সময় ডেলিভারী পদ্ধতিটি কি ছিল? (১= স্বাভাবিক, ২= সিজারিয়ান, ৩= ফরসেপ, ৪= অন্যান্য, ৫= প্রযোজ্য নয়) ☐

(খ) আপনার সর্বশেষ বাচ্চার জন্মের সময় কে সহায়তা করেছেন? (1= স্বাস্থ্যসেবা দানকারী কর্মী, 2= ডিগ্রীধারী ডাক্তার, 3= নার্স/মিডওয়াইফ/প্যারামেডিক্স, 4= প্রশিক্ষণ প্রাপ্ত দাই, 5= স্বাস্থ্য সেবা সহকারী, 6= আয়া, 7= গ্রাম্য ডাক্তার, 8= আত্মীয়, 9= প্রতিবেশী, 10= বান্ধবী, 11= প্রযোজ্য নয়) ☐

(গ) সর্বশেষ শিশুটি কত মাস জন্ম নিয়েছে (কত মাস গর্ভধারণের পর জন্ম নিয়েছে) ..... মাস = ১; ২= প্রযোজ্য নয়

(ঘ) জন্মের সময় শিশুর ওজন কত ছিল? (১ পাউন্ড ৪৫০ গ্রাম) (1 = ... গ্রাম, 2= মনে নেই/জানিনা, 3= ওজন নেয়া হয়নি ৩= প্রযোজ্য নয়) ☐

(ঙ) জন্মকালীন সময়ে আপনার বাচ্চার কোন সমস্যা হয়েছিল কি? (1= হাঁ, 2= না) ☐

(চ) সমস্যা হয়ে থাকলে কি ধরনের সমস্যা হয়েছিল? .....

৫৯। **বিগত ২৪ ঘন্টায় শারীরিক কার্যাবলীর বিবরণ**

বিভিন্ন সময়	কার্যাবলীর ধরন	কার্য শুরুর সময়	কার্য শেষের সময়	কার্যাবলী কোড	মোট সময় (মিনিট)
ফযরের আযানের সময়					
ঘুম থেকে ওঠা					
জোহরের আযানের সময়.....					
আছরের আযানের সময়.....					
মাগরিবের আযানের সময়.....					
রাতের খাবারের সময়.....					
ঘুমানোর সময়					

৬০। ডায়াবেটিস সনাক্ত হবার পর আপনার জীবন যাপনের কোন পরিবর্তন হয়েছে কি? (১ =হাঁ; ২ =না) ☐
যদি হাঁ হয় কোন ধরনের পরিবর্তন হয়েছে .....

৬১। জৈব রাসায়নিক পরীক্ষা (Biochemical Investigation)

#	ডায়াবেটিক পরীক্ষা	১	২	৩	৪	৫	৬
1	রক্তে শর্করার পরিমান খাবারের পূর্বে						
2	রক্তে শর্করার পরিমান খাবারের ২ঘন্টা পর						
3	ইউরিনারী মাইক্রোএলবুমিন						
4	HBA_1c						
5	Blood Insulin level						
6	লিপিড প্রোফাইল						
	এল ডি এল						
	এইচ ডি এল						
	কোলেষ্টেরল						
	ট্রাইগি□সারাইড						
7	লিভার টেষ্ট						
	ALT						
	SGPT						
8	কিডনী সিরাম ক্রিয়েটিনিন						
9	সিরাম জিংক						

সাক্ষাতকার গ্রহণ কারীর নাম ও স্বাক্ষর: .....