FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE

EVALUATION OF SERUM TRACE ELEMENTS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Sıpan Askandar RASHEED ALMIZORI

Master's Thesis

DEPARTMENT OF CHEMISTRY
Biochemistry

JULY 2020

FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE

Department of Chemistry
Biochemistry

Master's Thesis

EVALUATION OF SERUM TRACE ELEMENTS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Author

Sipan Askandar RASHEED ALMIZORI

Supervisor

Dr. Öğrt.Üyesi Aysel SARI

JULY 2020
ELAZIĞ

FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

TÜRKİYE

Department of Chemistry
Biochemistry

Master's Thesis

Title: Evaluation Of Serum Trace Elements in Patıents With Type 2 Diabetes Mellitus

Author: Sipan Askandar Rasheed ALMIZORI

Submission Date: 20 July 2020
Defense Date: 20 August 2020

THESIS APPROVAL

This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Firat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

		Signature
Supervisor:	Dr. Öğr.Üyesi Aysel SARI Firat University, Faculty of Science	Approved
Member:	Prof. Dr. Mustafa KARATEPE Firat University, Faculty of Science	Approved
Member:	Dr. Öğr.Üyesi Alpaslan KOÇAK Bingöl University, Faculty of Science	Approved
Member:	Title Name SURNAME ... University, Faculty of	Approved
Member:	Title Name SURNAME ... University, Faculty of	Approved

This thesis was approved by the Administrative Board of the Graduate School on

..... / ..... / 20 .....

Signature

Prof. Dr Soner ÖZGEN
Director of the Graduate School

DECLARATION

I hereby declare that I wrote this Master's Thesis titled “Evaluation of Serum Trace Elements In Patients with Type 2 Diabetes Mellitus” inconsistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

10.07.2020

Sipan Askander Rasheed ALMIZORI

PREFACE

My study is about the evaluation of serum some trace elements such as Mg, Fe, Cu, and Zn in patients who have T2DM and their roles in the regulation of T2DM. Because diabetic patients cannot stay fast for long hours, this became a hindrance for me to take some samples and there are some others who did not agree to take their samples because they did not have enough time or did not believe in this study, or any others reasons, so this took me a long time to collect enough samples. It took me a long time to obtain the materials and reagents for the study tests and this is the most important difficulty in my study.

First of all, I think God who gave me the strength and health and facilitated the ways for me to carry on my study.

I am extremely grateful and indebted to my supervisor Dr. Öğrt.Üyesi Aysel SARI for her concern, close supervision, constant encouragement and generous devotion of her time and advice, which guided me throughout my study and enable me to complete it.

I would like to express my sincere thanks to the laboratory Staff in Duhok Emergency teaching hospital where I previously worked on their laboratories machines. And the Staff of Duhok Diabetes centre especially Dr Idress for their unlimited help and support.

My deepest thanks are due to my friend Kawar Farsat Ali for his help in organizing parts of this work.

Finally, I am forever grateful to my parents, my wife, my brother, and sisters and all my friends for their tolerance and patience throughout this study and to all those who helped me in one way or another.

Sipan Askandar RASHEED ALMIZORI

ELAZIĞ, 2020

TABLE OF CONTENTS

Page

ABSTRACT

Evaluation Of Serum Trace Elements in Patients With Type 2 Diabetes Mellitus

Sipan Askander Rasheed ALMIZORI

Master'sThesis

FIRAT UNIVERSITY
Graduate School of Natural and Applied Sciences

Department of Chemistry
Biochemistry
July 2020, Page: xii+51

Background: T2DM is a widespread health issue where resistance to insulin action can result in an increased blood level of glucose. In the last decades, the prevalence of T2DM has dramatically elevated. Several research groups had reported that trace elements like Fe, Zn, Mg, and Cu may be involved in DM pathophysiology. These essential elements may be also involved in the development of vascular diseases and neuropathy usually seen in diabetic patients through binding to Glycated proteins.

Aim: To evaluate the serum Zn, Cu, Mg, and Fe in patients with T2DM and healthy controls. And to assertion the relation between HbA1c and trace element in T2DM.

Materials and Methods: This cross-sectional study was carried out in the Endocrine and Diabetes Unit at Azadi Teaching Hospital, in the Duhok city in Iraq. This study was performed in 92 subjects, 70 were T2DM patients and 22 were non-diabetic subjects. Mg and Fe were measured by using the autoanalyzer Cobas C 311. Cu and Zn were measured by autoanalyzer Biolis 24i, and HbA1c was done by (HPLC D10) autoanalyzer device.

Results: Zn, Mg and Fe concentrations were significantly lower in patients with T2DM than those without diabetes. In contrast, Cu in serum was significantly higher in T2DM than those without diabetes. Our study was also reported to have a significant positive correlation between Cu and HbA1c and a negative correlation between Mg, Zn and Fe with HbA1c.

Conclusion: Patients with T2DM had altered metabolism of Zn, Mg, Fe, and Cu. this may be related to the increased level of HbA1c. Impaired metabolism of these trace elements may have a contributory role in the progression of DM and its complications.

Keywords: Magnesium, Iron, Zinc, Copper, T2DM, Trace Element.

ÖZET

Tip 2 Diabet Mellitus İle Hastalarda Serum İz Eleman Elemanlarinin Değerlendirilmesi

Sipan Askander Rasheed ALMIZORI

Yüksek Lisans Tezi

FIRAT ÜNİVERSİTESİ
Fen Bilimleri Enstitüsü

Kimya Anabilim Dalı
Biyokimya
Temmuz 2020, Sayfa: xii+51

Arka fon: Tip 2 diyabet insulin etkisine karşı direncin arttığı yaygın bir sağlık sorunudur. T2DM prevalansı son yıllada önemli ölçüde artmıştır. Birkaç araştırma tarafından grubu Fe, Mg, Zn, ve Cu gibi eser elementlerin diyabetes mellitus patofizyolojisinde rolü olduğu düşünülmektedir. Diyabetik hastalarda bu elementler genellikle glikatlı proteinlere bağlanarak görülen vasküler hastalıklar ve nöropatolojiye neden olmaktadır.

Amaç: Bu çalışmada T2DM ve sağlıklı control grubu ile hastaların serumunda Cu, Zn, Fe and Mg T2DM'deki HbA1c ve eser element arasındaki ilişki değerlendirildi.

Gereç ve Yöntem: Bu kesitsel çalışma Irak'ın Duhok şehrinde bulunan Azadi Eğitim Hastanesi Endokrin ve Diyabet Ünitesinde gerçekleştirildi. Bu çalışma 92 denekte, 70'i T2DM hastası ve 22'si diyabetik olmayan sağlıklı kişilerde gerçekleştirildi. Serum Fe, Mg oto analizör Cobas C311 kullanılarak ölçüldü. Serum Cu ve Zn, otoanalizör Biolis 24i le ölçüldü ve glikatlı hemoglobin, (HPLC D10) otoanalizör cihazı ile yapıldı.

Bulgular: T2DM'li hastalarda Zn, Mg ve Fe konsantrasyonu diyabetli olmayanlar dan anlamlı derecede düşüktük olduğug özlendi. Aksine, serumda bakır T2DM'de diyabetli olmayanlara göre anlamlı derecede yüksekti. Çalışmamız ayrıca bakır ve HbA1c arasında anlamlı pozitif korelasyon ve Mg, Zn, Fe HbA1c arasında negative korelasyon olduğu rapor edildi.

Sonuç: T2DM hastalarında çinko, demir, magnezyum ve bakır metabolizması modifiye edildi ve bu, glisin hemoglobin seviyelerinin artmasıyla ilişkili olabileceği düşünülerek, bu eser elementlerin metabolizmanın bozulmasında ve komplikasyonlarının ilerlemesinde katkıda olabileceği düşünülektedir.

Anahtar Kelimeler: Magnezyum, Demir, Çinko, Bakır, T2DM, Eser element.

LIST OF TABLES

Page

LIST OF APPENDICES

ABBREVIATIONS

ADA	American Diabetes Association
AR	Average Dietary Requirements
ATP	Adenosine Tri Phosphate
BMI	Body Mass Index
CAD	Coronary Artery Disease
Cu	Copper
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DMT-1	Divalent Metal Transporter-1
EDTA	Ethylene Diamine Tetra Acetic Acid
FBS (FPG)	Fasting Blood (Plasma) Sugar (Glucose)
Fe	Iron
FNB	Food and Nutrition Board
GDM	Gestational Diabetes Mellitus
GLP-1	Glucagon-Like Peptide-1
HbA1c	Glycated Hemoglobin
HDL-C	High-Density Lipoprotein-Cholesterol
HH	Hereditary Hemochromatosis
ID	Iron Deficiency
IDF	International Diabetes Federation
LDL-C	Low-Density Lipoprotein-Cholesterol
Mg	Magnesium
NNR	Nordic Nutrition Recommendations
OGTT	Oral Glucose Tolerance Test
PAD	Peripheral Artery Disease
PTH	Para Thyroid Hormone
RDA	Recommendation Daily Allowance
Ris	Recommended Intakes
ROS	Reactive Oxygen Species
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus

TG	Triglyceride
WC	Waist Circumference
WHO	World Health Organization
Zn	Zinc

1. INTRODUCTION

Diabetes is a chronic condition that develops when the pancreas does not create enough insulin or when the body is unable to use the insulin it makes efficiently. Worldwide the prevalence of diabetes in age groups was estimated to be 8.3 % in 2013 and 10.1 % in 2035 between 20-70 years. According to IDF (International Diabetes Federation), the whole number of adults with diabetes is projected to develop to 592 million in 2035 from 382 million in 2013. Approximated 5.1 million humans have perished in 2013 from the effects of hyperglycemia. In low and middle-income nations, more than 80 % of diabetes deaths happen [1]. The ethnological forms of glycemic disorders include T1D (Type 1 diabetes), T2D (Type 2 diabetes), and GDM (gestational diabetes mellitus), T2DM is the prevalent type of diabetes and is characterized by complications of insulin action and secretion, both of which may be dominant characteristic [1]. Chronic hyperglycemia is the main point of diabetes mellitus which affects protein, fat, and carbohydrate metabolism disruption. While diabetes mellitus is classed as a single disease, there may be multiple secondary complications such as renal failure, heart defects, neuropathy, diabetic retinopathy, atherosclerosis, among others, the aetiology and complications of diabetes are still not clear, but many factors such as oxidative damage, ageing, and obesity have been involved [2].

The trace element is a dietary metal, essential for the organism's proper growth, evolution, and physiology. Changes in trace element status may have been due to chronic unregulated hyperglycemia, the human body needs them in extremely small quantities (in general < 100mg daily) [1].

Magnesium (Mg) is an important ion involved in the secretion and binding of insulin and its function at multiple levels; also it is a vital cofactor of several enzymes in the metabolism of carbohydrates. The typical magnesium level from 1.8-3.0 mg/dl, Mg plays an essential role in improving insulin resistance. A randomized controlled experiment showed that supplementation of oral Mg could boost insulin sensitivity even in subjects of normal Mg status who are not diabetic, this highlights the need to target Mg intake early to avoid insulin resistance and eventually T2DM. The probably increased incidence of hypomagnesaemia among T2DM patients is multifactorial. Contributing factors may be impaired insulin metabolism, poor glycemic regulation and osmotic diuresis. Diabetes hypomagnesaemia is typically seen in patients with impaired metabolic regulation or associated with DM complications according to clinical and epidemiological researches, the mechanisms in charge of Mg deficiency in diabetes patients have not yet been explained, especially regarding the effect on insulin resistance and diabetes progression and its continued complications [1].

Cu is known as both an effective catalyst for enzymes and a harmful reactant that creates hydroxyls radical, the normal amount of total Cu in the body from 70-140 μg/dl, a Cu deficiency contributes to glucose resistance, decreased insulin reply and increased glucose reply, this is linked with arteriosclerosis and hypercholesterolemia. Cu has an expression similar to insulin and facilitates lipogenesis, the latest studies showed that there are no significant differences in Cu levels between diabetic patients and stable subjects [1].

Zinc is a vital micronutrient that plays a significant role in many enzymes and serves as an effective antioxidant, zinc plays a part in the production, secretion and storage of insulin by pancreatic cells, the zinc content of the diabetic pancreas has been recognizing to be lower than that of the normal pancreas, besides zinc is recognized to play a role in insulin signalling as zinc deficient laboratory animals are much less sensitive to insulin, zinc can promote power consumption in skeletal muscle and brown adipose tissue, zinc also can enhance pancreatic insulin content and enhance glucose tolerance [3].

Iron is a transitional mineral and a powerful prooxidant that catalyzes many cellular reactions resulting in the formation of ROS (Reactive Oxygen Species), thereby raising the degree of oxidative stress. It leads to damaged tissue that may rais the risk of T2DM. In this regard, inherited hemochromatosis (HH), a genetic condition characterized by unusually high levels of circulating ferritin, is associated with an increased incidence of T2DM. However, people with T2DM have higher circulating levels of ferritin relative to healthy subjects, ferritin levels positively correlated with circulating levels of insulin and glucose, dyslipidemia, hypertension, and obesity, besides, many epidemiological studies recorded a positive correlation with elevated rates of ferritin, far lower than those found in (HH), and the risk of developing T2DM, and other associated conditions such as GDM, metabolic syndrome, probably CVD, and polycystic ovarian syndrome [4].

1.1. Aim and objectives of the study

1. To evaluate the serum Fe, Zn, Cu, and Mg in patients with T2DM and healthy controls.

2. To assertion, the relation between HbA1c and trace element in Type 2 diabetes mellitus.

3. The relation between age, smoking, genders, body mass index, physical activity, central obesity in a control subject and T2DM.

1.2. Type 2 Diabetes Mellitus (T2DM)

T2DM is a metabolic condition which is usually the product of excess caloric consumption over energy expenses. It is defined by insulin secretory defect due to insulin resistance which raises the body insulin demand to maintain homeostasis of glucose.

If β-cells fail to secrete enough insulin to balance the increased demand for insulin, blood glucose levels will gradually increase. Hyperglycemia is linked with long-term injury, deterioration and failure of various organs, in particular of the kidneys, blood vessels, heart, eyes, and nerves, resulting in increased mortality and morbidity. Type 2 diabetes linked with poor lifestyle is mainly a factor that contributes to progressive physical activity reduction and improvement in dietary habits. This would result in a larger percentage of the population being obese and overweight. Type 2 diabetes is one of the worlds most prevalent chronic diseases, and one of the 21st century's biggest public health issues [5].

1.2.1. Epidemiology of T2DM

The diabetes mellitus epidemic and its causes pose a significant risk to global health, the IDF estimated that in 2015, 1 in 11 adults aged 20 to 79 years (415 million people) worldwide had DM. This number is expected to grow to 642 million peoples by 2040, and the largest rises will come from the low-income and middle-income regions undergoing economic transitions. Such projections, however, may have under-represented the actual global burden of DM, especially in regions experiencing rapid epidemiological changes, the reasons for the growing epidemic of DM are numerous, including population ageing, urbanization, economic growth, sedentary lifestyle, and unhealthy eating habits. More than 90% of cases of DM are T2DM, good evidence suggests that many cases of Type 2 diabetes mellitus may be avoided by maintaining healthy body weight, exercising at least 30 min/day, adopting a balanced diet, consuming alcohol in moderation, and avoiding smoking [6].

1.2.2. Diagnosis of T2DM

Hyperglycemia is a major symptom of T2DM. Many common T2DM signs include polydipsia, polyuria, weakness, glucose to the urine, and weight loss, diabetes is usually diagnosed using the criterion for plasma glucose. FBG (Fasting Blood Glucose) and OGTT (Oral Glucose Tolerance Test) are the most commonly known diagnostic tests for T2DM. Diagnostic tests are widely used for both FPG (diagnosis of diabetes at plasma glucose levels of 126 mg/dl) and 2-hour OGTT (diagnosis of plasma glucose levels of 200 mg/dl). The benefits of FBS are low cost and the popularity of available automated laboratory machines. Although the oral GTT has long been identified as one of the diagnostic modalities for diabetes compared to FBS, in the

clinical setting it is less practical than an FBS test. In reality, the WHO has discouraged the usage of oral GTT for diabetes diagnosis because of its inconvenience, poor reproducibility and high cost [5]. For the following purposes, blood HbA1c is a good diagnostic tool. First, measurements of HbA1c can be performed at any time, and do not require planning by subjects studies. Secondly, HbA1c biological variation is low intra-individual, hence with high reproducibility, it is not affected by unexpected glycemic and psychological stress variations. Thirdly, HbA1c describes the mean levels of blood sugar over the last three months, it can, therefore, be assessed approximately every three months to assess whether patients glycemic control goals have been achieved and maintained. Fourth, epidemiological analyzes have estimated that a 25 per cent decrease in diabetes-associated death, a 35 per cent decrease in the risk of microvascular complications and an 18 per cent decrease in combined fatal and non-fatal myocardial infarction occurs with each percentage point decrease in HbA1c levels [5]. The ADA (American Diabetes Association) proposed HbA1c as a diagnostic criterion for diabetes in 2010, while the ADA recommended results of 6.5 % as the cutoff value for Type 2 diabetes mellitus diagnosis, determined by the production of diabetic retinopathy, which increases steeply at $\geq$ 6.5 % [5].

1.2.3. Complication and risk factors of T2DM

T2DM distinguishes two types of risk factors (modifiable and non-modifiable), modifiable risk factors include diets that are rich in simple carbohydrates and saturated fats, decreased glucose tolerance, high blood pressure ($\geq$ 140/90 mmHg), metabolic syndrome, elevated plasma triglycerides ($\leq$ 250 mg/dl), and poor physical activity (< 3 days in a week). Non-modifiable risk factors include age, family history of diabetes, race, and GDM. T2DM can cause severe complications if not adequately managed, most of these complications are linked to microvascular diseases for example (neuropathy, retinopathy, and nephropathy) and macrovascular diseases for example (Cerebrovascular disease, PAD, and CAD). While it is very necessary to regulate blood glucose level in diabetic patients, it is also essential to manage high blood pressure and dyslipidemia to avoid risks of the brain and cerebro cardiovascular. In T2DM patients, the risk of stroke increases by 150-400 %, while the risk of stroke-related dementia increases to (> 3) folds, metabolic changes such as dyslipidemia, insulin resistance and hyperglycemia ultimately contribute to atherosclerosis through the endothelial cell and vascular smooth muscle dysfunction combined with abnormal coagulation and impaired platelet function [7].

1.3. Iron

Nearly all cells, with slight exceptions, use Fe as a cofactor for essential biochemical activities such as synthesis of DNA, energy metabolism, and oxygen transport, this is due to the versatile chemistry of coordination and redox reactivity of Fe, allowing it to interact with proteins and connected it to oxygen, transfer electrons or mediate catalytic reactions [8].

Still, Fe is also possibly poisonous, as it catalyzes the propagation of reactive oxygen species and the production of highly reactive radicals such as hydroxyl radical by Fenton chemistry under aerobic conditions, as Fe switches easily between the reduced Fe^{+2} and oxidized Fe^{+3} forms, disruption of the cellular redox equilibrium need only a catalytic amount of the mineral, the resulting oxidative stress is linked with damage to cellular macromolecules, tissue injury and disease, it should also be noted that oxidized Fe^{+3}, given it is high abundance, is poorly bioavailable due to its limited solubility, the addition, regulation and detoxification of Fe, therefore, presents a major challenge for cells and organisms that have developed complicated mechanisms to fulfil their metabolic needs and reduce the hazard of toxicity [8]. The vast majority of body Fe at least 2.1 grams is distributed in red blood cell haemoglobin and produces erythroid cells, which assists in the transport of oxygen, there are also large concentrations of Fe in macrophages (> 600 mg), and muscle myoglobin (> 300 mg), while excess body Fe (> 1 gram) is contained in the liver. Mammals lose Fe due to sloughing of skin and mucosal cells or through bleeding but have no controlled mechanisms for body Fe excretion, while the close regulation of dietary Fe absorption in duodenum preserves the balance [8].

1.3.1. Dietary source of Fe

Fe metal is vital to health. Too little Fe can cause anaemia from Fe deficiency, so this can cause you to feel exhausted and raise your chance of disease or illness. Anaemia is fairly common, it's seen most frequently in children, teenagers and the elderly. Fe needs change according to sex and age, through periods of development (pregnancy, puberty, childhood and adolescence), and for menstruating women, the need for Fe increases. More Fe may also be required for preterm infants [9]. Two types of Fe are available: first one heme Fe originates from animal sources such as sheep, pork, beef, turkey, chicken and fish. The human body absorbs heme Fe better than non-heme Fe, the second type of Fe is non-heme this type is present in products fortified with grain, eggs, peas, beans, and vegetables, and some fruits. The body does not absorb all heme and non-heme Fe. When you are a vegetarian diet, you can need about twice as enough Fe in your diet, as your body just doesn't absorb the non-heme Fe, therefore you should strive to eat most meals with Fe-rich foods [9].

1.3.2. Recommendation of daily intake of Fe

The amount of Fe that body needed each day depends on the age, sex, and whether of eating a diet mostly based on plants, daily recommended amounts are shown that from birth to 6 months is 27 mg, infants from 7-12 months are 11 mg, children from 1-3 years are 7 mg, children from 4-8 years 10 mg, children from 9-13 years 8 mg, teen boys from 14-18 years 11 mg, teen girls from 14-18 years 15 mg, adult men 19-50 years 8 mg, adult women 19-50 years 18 mg, adults more than 51 years old needed 8 mg, pregnant women 27 mg, breastfeeding teens needed 10 mg, and breastfeeding women needed 9 mg. Vegetarians who do not eat meat, seafood, or poultry need almost twice as much as Fe because the body does not absorb non-heme Fe in plant foods as well as heme Fe in animal foods [10].

1.3.3. Functions of Fe

Although Fe comprises just 0.008 per cent of the body's mass (about 6 g for an adult male of 160 lb (75 kg), it is essential for our survival. Fe complexed with the protein haemoglobin is required to transport oxygen to the blood, remember that Fe is the heme group's central atom, a mineral complex that binds molecular oxygen in the lungs and transfers it to all other cells in the human body that require oxygen to conduct their activities (e.g. muscle cells), besides haemoglobin. Many essential proteins in the body include heme groups, including myoglobin, which extracts and transfers oxygen from haemoglobin to muscle cells, and cytochromes, which are important for energy generation, other proteins, such as those required to synthesize DNA and divide cells, also depend on Fe, besides, Fe helps to create the connective tissues in our body, strengthen the immune system and some of the neurotransmitters in our brain [11].

1.3.4. Clinical signification of Fe metabolism

Fe deficiency is one of the most common nutritional deficiencies in the world and affected about two billion peoples.

Many of these peoples are extremely Fe deficient that the production of RBC is impaired, hence causing into anaemia. The main cause of ID (Iron Deficiency) is the inadequate dietary intake, and poor Fe bioavailability in a plant-based diet increases susceptibility, in rare cases, the genetic disturbances in Fe homeostasis can result in ID, mutations in the TMPRSS6 gene encoding an upstream hepcidin regulator cause Fe refractory ID anaemia. Mild ID care can be done easily through increasing the amount of bioavailable Fe in the diet system for example (by raised of red meat Fe eating) or through using supplements. Commonly used Fe supplements, including ferrous gluconate, ferrous fumarate, and ferrous sulfate, can efficiently replenish a

depleted Fe supply but can cause serious side effects of the gastrointestinal tract at high doses [12].

Fe toxicity

Fe overload causes the damage of heart, liver, pancreatic, central nervous system to the tissue and thyroid, this organ damage is primarily caused by the overproduction of reactive oxygen species in the presence of excess Fe. The development of reactive oxygen species by Fe is primarily through the Fenton reaction, which ultimately produces hydroxyl radicals from hydrogen peroxide or superoxide. The hydroxyl radicals are the most toxic fraction among ROS and target carbohydrates nucleic acids and proteins. It is recognized that the reaction of hydroxyl radicals to nucleic acid-base 8-hydroxyguanine is strongly associated with oxidative stress with carcinogenicity and teratogenicity. The lipid hydroxyl-peroxide is another strong ROS exhibiting similar reactivity to the hydroxyl radical (ROOH), in Fe overload, lipid peroxidative products such as malondialdehyde, which form the RO-(radical alkoxy) and ROO-(alkyl oxyradical) radicals, are increased, these lipid-based radicals have longer half-lives than hydroxyl radicals and are also more capable of DNA damage and chronic cell toxicity [13].

1.3.5. Fe and type 2 diabetes mellitus

Data proof that systemic Fe overload may lead to abnormal glucose metabolism was first derived from the observation that in classical hereditary hemochromatosis (HH) the risk of diabetes is increased, nevertheless, with the discovery of novel Fe metabolism genetic diseases, it is evident that Fe overload, regardless of the problem or gene affected, contributes to an enhanced incidence of T2DM, the role of Fe in DM is indicated by an enhanced incidence of T2DM in various causes of Fe overload, and diabetes reversal or improvement of diabetes (control blood sugar level) with a decrease in Fe amount obtained by phlebotomy or Fe chelation treatment, a connection has recently been identified between increased dietary intake of Fe, notably eating red meat and increased body Fe stores and diabetes growth, improved insulin sensitivity and insulin secretion with regular blood donation and reduced Fe stores indicate a causative link with Fe overload studies in patients with thalassemia indicate a large rise in insulin resistance [14]. During the research of patients with unexplained hepatic high Fe, the majority were found to be insulin resistant, indicating a common etiological correlation between liver dysfunction, hepatic Fe, and insulin resistance [14].

1.4. Magnesium

Mg is the fourth most common element in the body, following calcium, potassium, and sodium, and the second most prevalent intracellular cation after potassium [15].

Mg is mainly present inside the cell where the energy-rich ATP and nuclear acids serve as a counter ion. Mg^{+2} is a cofactor in higher than 300 enzyme systems that improve various biochemical responses in the body, including blood pressure regulation, blood glucose controller, signal transduction, neuromuscular conductivity, muscle and nerve transmission, and protein synthesis [16].

1.4.1. Physiology of magnesium

Less than one per cent of total Mg can be stored in serum and red blood cells in humans, it is primarily distributed between the bone about 53 % and the muscle intracellular compartments 27 % and the soft tissues 19 % [17]. 90 % of the magnesium intracellular is bounded-to organic matrices. Serum Mg contains just 0.3 % of the total body Mg in three cases, ionized 62 %, protein-bound 33 %, chiefly to albumin, and 5 % complexed to anions such as phosphate and citrate. For most radiolabel-led magnesium, the equilibrium between tissue pools is given gradually with a half-life ranging between 41-181 days. Consequently, serum Mg figures can not give characteristic data about the condition of other stocks [17].

1.4.2. Dietary source of Mg

Mg dietary deficiency of magnitude enough to induce pathological changes is rare, Mg is commonly dispersed in plant and animal feeds, and geochemical and other environmental factors seldom affect its food quality. Nuts, most green vegetable, peas, legume seeds, and beans are rich in Mg, as are some soy flour, spices, and shellfish, all typically containing > 500 mg/kg of fresh weight. Although most unrefined cereal grains are reasonable sources, mushrooms, many highly refined flours, fruits, and tubers, and most fats and oils contribute little dietary Mg < 100 mg/kg fresh weight. Cornmeal, sago, and cassava meal and polished rice meal have an exceptionally low Mg content [18].

1.4.3. Recommendation of daily intake of Mg

Dietary studies of people in Europe and the US more show that Mg intakes are lower than the required amounts. Epidemiological researches in North America and Europe have explained that people who use Western-type diets system are poor in Mg content, i.e. less than 30-50 % of the Mg RDA, it is proposed that Mg dietary eating in the US has decreased over the last 100 years

from around 500 to 175-225 mg/day. The institute of medicines FNB (Food and Nutrition Board) raised the intake of dietary recommendations (RDA) for Mg in 1997, dependent on the finding of controlled balancing studies, the latest RDA ranges from 80 mg/day for 1-3 years old children to 130 mg/day for 4-8 years old children. About the older males, Mg RDA ranges from as low as 240 mg/day range, ages 9 to 13 and rises to 420 mg/day for men ages 31 to 70 years and older. With females, Mg RDA reaches from 240 mg/day at the ages 9-13 to 360 mg/day for females at the age of 14-18. The RDA for women aged 31-70 and over are 320 mg/day [16].

1.4.4. Functions of Mg

Mg is present mainly in the cell that it serves as a counter ion for the energy-rich ATP and nuclear acids. Mg is a cofactor in higher than 300 enzyme systems that improve the various biochemical reactions in the human body, including muscle and nerve transmission, protein synthesis, and blood pressure regulation, blood glucose control, signal transduction, and neuromuscular conduction, and many enzymes that are reliant on Mg include Na^+/K^+-ATPase, protein kinase, cyclases, hexokinase, and creatine kinase. Mg is also important for protein, nucleic acid or mitochondrial structural functions, it is required for the synthesis of RNA and DNA, and for the development of anaerobic and aerobic energy oxidative phosphorylation and glycolysis either indirectly as part of the Mg-ATP complex, or directly as an enzyme activator, Mg also works a crucial part in the active transfer of potassium and calcium ions through membranes of the cell, a vital mechanism for the conduction of nerve impulses, vasomotor tone, muscle relaxation and regular heart rhythm [16].

1.4.5. Clinical signification of Mg metabolism

Mg deficiency is not uncommon in the general population, it is consumption has been declining in the western world in particular over the years, hypomagnesaemia is characterized as the level of serum Mg < 0.75 mmol/l. Early symptoms of Mg deficiency include lack of appetite, vomiting, nausea, lethargy, tiredness and fatigue, more obvious deficiency in Mg shows symptoms of increased neuromuscular excitability like generalized seizures, tetany, tremor, muscle cramps, and carpopedal spasm. Also, hypomagnesemia, including atrial and ventricular tachycardia, can cause cardiac arrhythmias.

Mg deficiency

Mg deficiency is also linked with other irregularities in the electrolytes, such as hypocalcaemia and hypokalaemia. The conditions that may contribute to Mg deficiency include alcoholism, poorly regulated DM, malabsorption for example (ulcerative colitis, Whipple's

disease, Crohn's disease, short bowel syndrome, coeliac disease), endocrine causes such as (Hyperthyroidism, Hyperparathyroidism, Alosteronism), a renal disease for example (syndrome of Gitelman, dialysis, chronic renal failure), and drug usage Mg deficiency can be caused by some medications including proton pump inhibitors, antibiotics, diuretics, and chemotherapeutic agents[16].

Mg toxicity

Hypermagnesemia is characterized as the blood level of an abnormally elevated Mg^{2+}. Hypermagnesemia is typically the results of an excess of Mg in the human body, whole-body homeostasis Mg^{+2} is the product of the balance of absorption in the intestine, storage in bones, and excretion by kidneys. Therefore, hypermagnesaemia is usually the result of disease in any of these parts. Since the tow kidneys excrete excess Mg^{+2} very efficiently, hypermagnesemia occurs seldom and is mainly observed when the renal creatinine clearance falls < 30 ml of urine in one minute. Hypermagnesemia, therefore, occurs almost exclusively in patients with renal failure who receive Mg salts or Mg^{+2} containing medications such as laxatives or antacids and is typically concomitant with hyperkalemia and/or hypocalcemia. The main symptoms are hypotension, impaired breathing, muscle weakness, decreased or absent deep tendon reflexes, arrhythmia, and bradycardia as a result of abnormal nervous, muscular and cardiac electrical conduction. High concentrations of Mg are linked with vomiting and nausea in an attempt to restore electrolyte levels. Since some of these symptoms arise after the onset of hyperkalemia and/or hypocalcemia, it is difficult to assess the degree of which one of these 2 conditions leads to the appearance to the symptoms [19].

1.4.6. Mg and T2DM

The relation between Mg deficiency and T2D is well known, T2DM is often linked with both intracellular and extracellular depletion of Mg, especially in patients with poorly regulated glycemic profiles, longer disease duration, and micro- and macrovascular complications. Between a low dietary input of Mg and an elevated urinary loss of Mg can work Mg exhaustion in diabetic patients, a reversed correlation was identified between dietary Mg and the incidence of T2DM, insulin resistance and an enhanced risk of increasing glucose intolerance and T2DM are associated with a diet that poor in Mg. It was suggested to use Mg supplements as a possible method for T2DM prevention and metabolic regulation. Benefits of Mg supplements on glycemic profile have been observed in several studies but not all. Work on Mg supplementation in individuals with diabetes or at risk of it is inadequate [20].

1.5. Zinc

Zinc (Zn) is the 2nd most abundant trace element, important for all living organisms after Fe. Zn^{2+} survives as divalent cation and is not redox-active under physiological conditions which explains why Zn performs multiple physiological roles in a variety of biological processes.

Zn is redox neutral, and is reactive in biological reactions as lewis acid, unlike Fe and Cu. These features indicate that Zn plays key roles as structural, signalling, and catalytic component. Zn is an essential trace mineral for life, Zn is significance to living organisms was first recognized in Aspergillusniger in 1869, Afterwards, Zn was found to be important for normal plant development and normal rat and bird growth. Zn was not however recognized as an important micronutrient for humans until 1961 [21].

1.5.1. Dietary source of Zn

Foods including high proteins content are also rich in Zn, whereas those mainly carbohydrates foods and diets were found to be much lower in Zn content. Meat-derived foods account for a high percentage of Zn between 0.4-6.77 mg/100g, the grain category has between 0.3-2.54 mg/100g, dairy products between 0.36-0.49 mg/100g, vegetables between 0.12-0.6 mg/100g, and fruits have between 0.02-0.26 mg/100g. Foods of animals origin are the primary human dietary sources of Zn, the oyster is rich in complexes of Cu and Zn, lean red meat, red muscle meat from the poultry, turkey meat, and beef liver are also healthy sources of Zn. Zn is also supplemented by skimmed condensed milk, cheddar cheese, and egg. Since protein intake in vegetarians is mainly through pulses and not meat, they have a higher incidence of Zn deficiency. Intake of cereals, legumes, starchy roots such as potatoes can contribute to lower bioavailability of Zn. Due to the greater phytate content in porridge, the amount of Zn absorption was found to be lower in porridge compared to bread. For patients with a Zn deficiency, vitamin A is also consumed and metabolized to a lesser degree [22].

1.5.2. Recommendation of daily intake of Zn

Zn requirements differ by age and bioavailability. Within the diet, some bioactive compounds such as phytic acids and tannins interfere with Zn absorption and thus increase the requirements for Zn, the criteria differ twenty fold depending on the stage of life and diet bioavailability. Adequate Zn nutrition is especially essential in children because of its function in cell division and differentiation [23]. In 2004, Nordic Nutrition Recommendations calculated the (AR) average dietary requirements for Zn to be 6.4 and 5.7 mg respectively for men and women, the recommended ıntakes (RIs) were set at 7 mg/day for women and 9 mg/day for men using an inter-individual criteria variance of 15 %. 2014 recommended ıntakes (RIs) were kept unchanged

in Nordic Nutrition Recommendation 2012, as no new scientific evidence has emerged justifying a change. The criteria for systemic Zn and endogenous losses for adolescents and children are higher than adults, hence the (RIs) per body weight for adolescents and children are higher than adults, these values were also reported in (NNR 2004) and have not been altered in the 2012 report due to no new data. The recommendation of daily intake of Zn is different in the age and sex that in the 6 months to 2 years are 5 mg, between 2-5 years 6 mg, 6-9 years 7 mg, and a male between 10-13 years is 11 mg/day, and female between 10-13 years 8 mg, and a male between 14-17 years 12 mg, and female in same years old 7 mg/day, and male in more than 18 years 9 mg/day, female more than 18 years 7 mg, finally female during pregnant and lactating are between 9-11 mg/day [23].

1.5.3. Functions of Zn

Zn, an important trace metal, is required for the metabolic activity of more than 300 enzymes of the human body and is considered necessary for cell division and DNA and protein synthesis, these enzymes are associated with carbohydrates, proteins, and fats metabolisms. Zn is also important for tissue development, taste acuity, wound healing, maintenance and development of connective tissue, the function of the immune system, production of prostaglandin, bone mineralization, blood clotting, proper thyroid functions, cognitive function, sperm production, and growth of the fetus, Zn is needed to maintain normal testosterone levels, Zn is located in the vesicles of the hippocampus of the brain mossy fibre system, Zn is important to the immune system and Zn deficiency has significant implications for immune functions, Zn supplementation has been shown to enhance the immune response of healthy elderly people through cell mediation. Zn is vital to healthy skin, Zn activates brain areas which receive and process information from sensors of odour and taste, Zn concentration in plasma have been found to affect appetite and taste preferences [24].

1.5.4. Clinical signification of Zn metabolism

The primary syndromes of Zn deficiency can be attributed to diets that are deficient in Zn.

Acrodermatitis enteropathica, characterized by a triad of dermatitis, alopecia and diarrhoea exists as an autosomal recessive condition due to gene mutations (SLC39A4), which symbolize ZIP4, the protein of the Zn transporter, it may also occur in an acquired form in exclusively breast-fed infants due to mutation in their mothers in the Zn transporter gene SLC30A2 (Zn T-2), which is responsible for the transfer of Zn from serum to breast milk. Secondary or programmed Zn deficiency occurs in a variety of conditions such as malabsorption syndrome, liver cirrhosis,

chronic renal failure, complete parenteral nutrition, sickle cell disease, diabetes, malignancies, other medical diseases and penicillamine, anticonvulsants and ethambutol drug therapy [25].

Zn deficiency induces various organs including the integumental, central nervous, gastrointestinal, immune, respiratory, reproductive systems, and skeletal. Zn deficiency induces both humoral and cell-mediated immunity dysfunction and increases the vulnerability to infection. Disorders in the metabolism of nucleic acids and the synthesis of proteins can account for certain features of Zn deficiency. A relative excess of secondary dietary nitrogen to Zn deficiency can cause anorexia and taste impairment. Zn status can affect growth hormone functions, thyroid, insulin functions, sex hormone, corticosteroid, gonadotropin, and prolactin. Development retardation happens because of disturbance of the role of insulin-like growth factor which interferes the cellular effects of growth hormone [25]. Slowed sexual maturation and impotence may occur, as may hypothermia and hypogonadism. Elevated prostaglandin synthesis, particularly PGE-2 that happens in Zn deficiency, can also lead to, alopecia, diarrhoea, nail dystrophy, acro-orificial skin lesions, and glossitis. Mental shifts were also observed in the form of apathy, depression and behaviour change. There is also delayed healing of wounds, burns and ulcers from the decubitus. Eye injuries have been reported including photophobia and lack of dark adaptation, corneal opacities, conjunctivitis, night blindness, and macular degeneration [25].

Zn toxicity

Zn toxicity due to chronic or acute ingestion of high Zn supplements can also occur, leading to compromised immune response, microcytosis, hypocupremia, neutropenia, and excess Zn intake can lead to lethargy and ataxia. The toxicity to Zn can occur in both chronic and acute forms. High Zn consumption has acute adverse effects including vomiting, nausea, stomach cramps, loss of appetite, headaches, and diarrhoea, intakes of 150-450 mg of Zn in a day were linked with chronic effects such as altered Fe functions, low Cu status, decreased immune functions, and decreased HDL levels [24].

1.5.5. Zn and Type 2 diabetes mellitus

Ninety per cent of diabetes patients have T2DM, defined by hyperinsulinemia, insulin resistance, β-cells dysfunction, and eventual β-cells failure. Insulin is processed as a hexamer containing 2 Zn ions in pancreatic β-cells and released into a portal venous system at a time of degranulation of β-cells. The Zn^{2+} ions co-secreted with insulin inhibit the monomeric insulin's intrinsic amyloidogenic properties. In vitro evidence indicates that insulin binds more closely to isolated liver membranes and that less degradation occurs when coadministered with Zn. Zn is required for most hormones, including insulin, are to work healthily. Adequate Zn plays at least three functions in the safety of insulins. Zn binds to insulin to properly store insulin in the

pancreas and to release it when glucose reaches the bloodstream. Zn enhances cell safety, constituting a part of the enzymes required to bind insulin to cells so glucose can go inside and be used as fuel. The cycle of insulin binding to the cell is what the term "insulin sensitivity" refers to and means the cell is sensitive to insulin. When insulin binds to the cell, it will "open the door" to allow the glucose to enter. If the cell is insulin resistance, glucose can remain in a bloodstream, cause raises in blood glucose and eventually result in lipid increasing, as Zn concentration reduces, insulin secretion and peripheral insulin sensitivity decrease, which, if persistent, leads to DM [26].

1.6. Copper

Copper (Cu) is an important trace metal element found in all living organisms in the states of oxidized Cu (II) and reduced Cu(I) [27].

The human body is only required in trace amounts and contains around 100 mg Cu. This is the cofactor of several redox enzymes as a transitional metal, ceruloplasmin being the most abundant Cu dependent ferroxidase enzyme with the Cu dependent oxidation activity. In addition to Cu involvement in Fe metabolism, the need for copper also derives from its functioning in a myriad of biological process, including neuropeptide synthesis, immune functions, and antioxidant defence, consequently, the wide variety of clinical symptoms arising from disturbances in cuproenzyme activities. Means it while severe Cu deficiency is fairly candid to diagnose, it is moderately troublesome to recognize marginal deficiencies [28].

1.6.1. Dietary source of Cu

Cu comes from a large selection of new and refined products. Better sources involve nuts, grains (cashew nuts and brazil in special), meat (kidney and liver), shellfish, legumes (beans and peas) and seeds. Dark chocolate is rich in a Cu source, too, eating a well-balanced diet would allow the body to satisfy its daily Cu needs [29]. Human and cow milk are low copper sources but breast milk has a higher Cu content than cow milk does. In comparison, the concentration of Cu in breast milk decreases with lactation time: colostrum and transitory milk have the highest values. Cu is added to most baby formulae. The amount of Cu in infant formulas varies according to the child's need (full-term or preterm) [30].

1.6.2. Recommendation of daily intake of Cu

Cu is an important trace mineral which the human body can not form so it must be consumed every day from dietary sources. According to the World Health Organization, 1-3

milligrams of Cu in a day is required to avoid any deficiency symptoms (adult 0.9, pregnant woman 1.0, nursing mother 1.3, child 9-13 years 0.7) mg/day. Different nutrition and health organizations throughout the world have set dietary guidelines, stressing the significance of Cu as part of a healthy diet [29].

1.6.3. Functions of Cu

Virtually every cell in the human body uses Cu. Cu is one of the trios of minerals important for good health, along with Fe and Zn. From embryonic development through to old age, Cu is important to the health of the human body, the human brains, nervous systems, and cardiovascular systems can not function normally without Cu [29]. Cu is also important for brain development during fetal and postnatal growth and life-long maintenance of brain health including effective antioxidative defence and effective nerve cell communication, good skincare and the connective tissue, healing wound, structural integrity, and blood vessel and heart work, development of the new blood vessel, proper structure of circulating blood cells and their function, cell composition of the immune system, maintenance of the safe and efficient immune response, generation and energy storage in our cells power plants, and the mitochondria [29].

1.6.4. Clinical signification of Cu metabolism

Since Cu is involved in many of the human body functions, its deficiency can produce a large array of symptoms.

Cu deficiencies may lead to hernias, aneurysms, bleeding of blood vessels or nosebleeds, ID anaemia, osteoporosis and joint problems, brain disorders, cholesterol and glucose metabolism abnormalities, increased susceptibility to infection due to impaired immune functions (neutropenia), pigment loss, fatigue, skin sores, tiredness, irregular heartbeat, impaired thyroid functions, and low body temperature. So Cu is essential in the structure of cell membranes [31].

Cu toxicity

Inordinate eating of Cu can cause vomiting, nausea, cramps, and abdomen pain, dizziness, headache, fatigue, diarrhoea, and a metallic taste in the mouth (related to water with concentrations of Cu more than 6 mg/l). Deliberately high Cu intakes cause renal and liver damage and also death. Chronic toxicity of Cu is not usually present in humans due to transport mechanisms that control excretion and absorption, because excess Cu is excreted by bile, the most likely incidence of Cu toxicity is in individuals with liver disease or other medical conditions in which bile excretion is compromised, it has still not been determined if Cu is carcinogenic or not. Postpartum depression was also associated with elevated Cu levels, it is

because Cu levels increase to around twice the usual values during the pregnancy, and it can take up to three months after delivery to normalize Cu levels, scientific studies are showing a correlation between long-term exposure to high Cu levels and drop in intelligence among young adolescents [31].

1.6.5. Cu and T2DM

The study of laboratory animals showed that Cu deficient rats appeared to have elevated levels of blood glucose over time, suggesting a potential correlation between low Cu and DM. Nonetheless, a clinical review that involved people with DM did report very different results. In people with diabetes, Cu levels were higher compared with those without DM. Indeed the higher level of Cu, more likely the person will have DM complications, like high blood pressure, vascular disease, and retinopathy [31].

2. MATERIALS AND METHODS

This cross-sectional study was carried out in the Endocrine and Diabetes Unit at Azadi Teaching Hospital and Emergency Teaching Hospital in the Duhok city in Iraq. The study spanned the period from September 2019 to September 2020 using convenient sampling techniques.

2.1. Subjects and study design

A total number of 70 of diabetic outpatients visiting the endocrine and diabetic unit were selected. Based on the diabetes record for each diabetic patient, the following conditions were excluded: patients with chronic kidney disease, patients with chronic liver disease, patients who are taking folic acid and multivitamin (A - Z), or vitamin B12 and Fe. Patients who are taking Zn and cupper and Mg supplements, patients that are injected with insulin, pregnant women, inflammatory conditions, and for patients with T1DM. In this study we take 92 subjects, 70 subjects are patients in T2DM (case group), and other 22 individual healthy (control group) were recruited by personal request from the staff of Azadi Teaching Hospital and Emergency Teaching Hospital in Duhok city, the blood samples will be collected after an overnight fast for the test.

The study group ages were between 35 and 75 years. Informed consent was obtained from each participant commenced before the study and the recruited one were approved by the ethics committee of the directorate of health, of Duhok city Governate.

A meeting interview was conducted for filling a questionnaire form that was designed to match the study needs. The questionnaire form was similar to the diabetic clinic questions at the Endocrine and Diabetic Unit with some modifications.

The questionnaire form includes questions such as name, age, sex, smoking habit, type of drugs taken for diabetes mellitus (only for patients), physical activity, duration of diabetes mellitus, and history of minerals taking (Mg, Fe, Cu, and Zn), with the calculation of body mass index.

2.2. Assessment of studied variables

Our partic pants vas under go to some sepical question to clearity our results

2.2.1. Ages groups

The subjects were classified according to their age into two groups:

1. Equal to, or less than 40 years.
2. More than 40 years.

2.2.2. Smoking habit

Based on smoking habits, the subjects were classified into two groups:

1. Non-smoker, when no cigarette was smoked daily.
2. Smoker (either light or heavy smoker) when more than ten cigarettes were smoked daily.

2.2.3. Duration of diabetes mellitus

Patients were classified depending on the duration of diabetes into two groups:

1. Equal to or less than 5 years.
2. More than 5 years.

2.2.4. Physical activity

According to physical activity subjects were classified into two groups:

1. Inert physical activity when the patient did not exercise or for less than 15 minutes daily.
2. Active physical activity when the patient did exercise for 15 minutes or more daily.

2.2.5. Body mass ındex (BMI)

1. BMI between 18.5-24.9 kg/m^2 was estimated as normal weight.
2. BMI between 25.5-29.9 kg/m^2 was estimated as overweight.
3. BMI equal to, or more than 30 kg/m^2 was estimated obese.

***BMI=Weight (Kg)/ (Height (m))^2**

2.2.6. Diabetes status (Glycemic control)

For glycemic control, glycated haemoglobin was measured for all diabetic patients [32]:

1. HbA1c less than 6.5% was considered good control.
2. HbA1c equal to, or more than 6.5% was considered fair control.

2.2.7. Lipid profile status:[33]

1. Total serum cholesterol

1. Total serum cholesterol < 200 mg/dl were considered as normal
2. Total serum cholesterol between 200-239 mg/dl was considered borderline.
3. Total serum cholesterol ≥ 240 mg/dl was considered hypercholesterolemia.

2. Serum triglyceride (S.TG)

1. S.TG < 150 mg/dl was considered as normal.
2. S.TG between 150-199 mg/dl was considered.

3. S.TG between 200-499 mg/dl was considered as hypertriglyceridemia.
4. S.TG ≥ 500 mg/dl was considered as a very high-risk factor.

3. Serum high density lipoprotein cholesterol (S.HDL-C)

1. S.HDL-C less than 40 mg/dl was regarded as a low level.
2. S.HDL-C equal to, or more than 40 mg/dl was considered as normal level.

4. Serum low density lipoprotein cholesterol (S.LDL-C)

1. S.LDL < 100 mg/dl was considered optimal.
2. S.LDL between 100-129 mg/dl was regarded as near-optimal.
3. S.LDL between 130-159 mg/dl was considered as a borderline high-risk factor.
4. S.LDL between 160-189 mg/dl was considered as a high-risk factor.
5. S.LDL ≥ 190 mg/dl was considered as a very high-risk factor.

2.2.8. Serum Mg level:[20]

1. S.Mg < 1.7 mg/dl was considered as a low level.
2. S.Mg between 1.7-2.5 mg/dl was considered as normal value.
3. S.Mg > 2.5 mg/dl was considered as high value.

2.2.9. Serum Fe level:[33]

1. Serum Fe level < 50 μg/dl was considered as a low level.
2. Serum Fe level between 50 and 150 μg/dl was considered as normal level.
3. Serum Fe level > 150 μg/dl were considered as high level.

2.2.10. Serum Zn level[33]**:**

1. S.Zn < 70 μg/dl was considered as a low level.
2. S.Zn between 70-140 μg/dl was considered as normal value.
3. S.Zn > 140 μg/dl was considered as high level.

2.2.11. Serum Cu level:[33]

1. Serum Cu level < 100 μg/dl was considered as a low level.
2. Serum Cu between 100 and 200 μg/dl was considered as normal value.
3. Serum Cu level > 200 μg/dl was considered as a high level.

2.3. Methods

70 blood samples were collected from T2DM patients diagnosed according to the WHO protocol. 22 blood samples were also collected from apparently healthy individuals (control group).

2.3.1. Collection and processing of blood sample:

Participants who attended the endocrine and diabetic unit in the morning were fasting overnight for 12 to 14 hours. Venous blood samples (6 ml) were collected between 8:30 -11:30 AM, withdrawn by venipuncture using vacutainer from the antecubital vein. Two ml were collected immediately into a vacuum tube containing K3 EDTA as an anticoagulant for estimation of HbA1c. The remainder 4 ml was collected in vacutainer system gel separator tubes.

The serum was separated from the whole blood after clotting using centrifugation process (HITACHI centrifuge, model O5P-21) at 5000 rpm for 10 min, the serum was processed immediately for measuring glucose, total cholesterol, triglycerides, HDL, LDL, Mg, Fe, Cu, and Zn.

2.3.2. Biochemical analysis

Most of the tests were performed by the Cobas c311 and Biolis 24i devices for laboratory analyzes, and here we review all these tests. **Appendix (1), (2)**

Fasting blood sugar

The sugar level is detected by using Glucose HK Gen.3 300 tests Roche (Hitachi) Cobas c 311, with reference number 04404483 190.

Cholesterol

The cholesterol level is detected by using Cholesterol Gen.2 400 tests Roche (Hitachi) Cobas c 311, with reference number 03039773 190.

Triglyceride

The triglyceride level is detected by using Triglyceride. 250 tests Roche (Hitachi) Cobas c 311, with reference number 20767107 322.

HDL

The HDL level is detected by using HDL-Cholesterol Gen.4 tests Roche (Hitachi) Cobas c 311, with reference number 07528566 190

LDL

The LDL level is detected by using LDL-Cholesterol Gen.3 tests Roche (Hitachi) Cobas c 311, with reference number 07005717 190.

Mg

The Mg level is detected by using Mg Gen.2 250 tests Roche (Hitachi) Cobas c 311, with reference number 06481647 190.

Fe

The Fe level is detected by using Fe Gen.2200 tests Roche (Hitachi) Cobas c 311, with reference number 03183696 122.

(www.roch.com 2013-10, v 3.0 English)

Zn

The Zn level is detected by using IMPROGEN description Zn Reagent 1-2 *25 ml Biolis 24i with reference number ZIN4911.**Appendix (2)**

Cu

The Cu level is detected by using IMPROGEN description Cu Reagent 1-2 *25 ml Biolis 24i with reference number CU04911. **Appendix (2)**

HbA1c

Determination of HbA1c level was done by HPLC D10 Instrument Bio-Rad.**Appendix (3)**

2.4. Statistical analysis

Data were analyzed using SPSS the statistical package for social sciences program version 23, T-test was used to compare proportions. P-value $\leq$ 0.05 was considered statistically significant, while P-value was 0.01 it considered statistically as a highly significant. One Way ANOVA was used to compare among more than two groups, the Pearson correlation was used to determine the correlation coefficient between study parameters was determined by using the Pearson correlation.

3. RESULTS AND DISCUSSION

The study considered 70 diabetic patients in comparison with 22 healthy controls. The study participants were 43 males and 49 females.

3.1. General characteristic of studied participants

The study includes 77 subjects 84 % of whom non-smokers, and out of 68 subjects, 74 % did not do exercise in comparison with 15 smokers 16 % and 24 physically active subjects 26 %. Furthermore, the mean age of the subjects and patients was (49.52 ± 9.93) years (Table 3.1). The mean ± SD of BMI was (29.72 ± 4.79 kg/m$^{2)}$. The mean of the patients that have diabetes mellitus during ≤ 5 years was (32), (45.7 %), and the (38), (54.3 %) in the case that has in > 5 years. In addition, the mean ± SD of biochemical indicators of Glucose, HbA1c, Mg, Fe, Cu, Zn, cholesterol, triglyceride, HDL, LDL levels were (156.72 ± 63.38 mg/dl), (7.96 ± 2.13 %), (2.09 ± 0.2 mg/dl), (88.14 ± 35.88 μg/dl), (99.27 ± 11.36 μg/dl), (79.07 ± 13.29 μg/dl), (176.93 ± 38.49 mg/dl), (185.38 ± 82.47 mg/d), (44.21 ± 10.43 mg/dl), (102.50 ± 36.78 mg/dl), respectively (Table 3.2).

Table 3.1. General characteristic of studied participants

Subject characteristics (n=92)	Frequency Distribution	
	Frequency or Mean, Median	Percentage Or S.D, IQ
Subject categorizes		
Control	22	24%
Diabetic	70	76%
Age (years)*	49.52	9.93
Gender* **		
Male	43	47%
Female	49	53%
BMI * (kg/m^2)	29.72	4.79
Duration of diabetes (years)***		
≤5 years	32	45.7%
>5 years	38	54.3%
Exercise**		
Yes	24	26%
No	68	74%
Smoking**		
Yes	15	16%
NO	77	84%

Table 3.2. General characteristics of biochemical indicators:

biochemical indicators	Frequency Distribution	
	Frequency or Mean, Median	Percentage Or S.D, IQ
Glucose (mg/dl)*	156.72	63.38
HbA1c (%) **	7.96	2.13
Mg (mg/dl)*	2.09	0.21
Fe (µg/dl)*	88.14	35.88
Cu (µg/dl)*	99.27	11.36
Zn (µg/dl)*	79.07	13.29
Cholesterol (mg/dl)*	176.93	38.49
Triglyceride (mg/dl)**	185.38	82.47
HDL (mg/dl)*	44.21	10.43
LDL (mg/dl)**	102.50	36.78

*mean and S.D for normal, **median and interquartile for non-normal data and ***frequency and percentage were performed in the calculations.

3.2. Patients and subjects characteristics according to control, and diabetic patients

With stratification of patients and subjects characteristic based on the study group, the study revealed that the majority of the patients and subject were females (14), (64 %) in the control group and (35), (50 %) diabetic patients, and males (8), (36 %) in the control group and (35), (50 %) diabetic patients with no statistically significant difference between the subjects genders (P= 0.398). Besides, the study revealed that most of the participants were non-smokers in the two studied group, (82 %), (84 %), control, and diabetic groups respectively (p=0.785), whereas the majority of the controls (64 %), and diabetic patients (54 %) were non-exercisers (p=0.208). The mean ± SD of age was found in the controls (41.22 ± 7.83) and diabetic patients (52.12 ± 9.09) with a statistically substantial difference (p<0.001). The mean ± SD of BMI, glucose, HbA1c, Cu, triglyceride, in diabetic patients, was greater than in the control subjects, BMI (kg/m^2) (30.50 ± 4.70) (p<0.01), Glucose (mg/dl) (176.64 ± 59.90) (p<0.001), HbA1c (%) (8.81 ± 1.71) (p<0.001), Cu (µg/dl) (100.60 ± 11.47) (p<0.05), and triglyceride (mg/dl) (203.24 ± 80.88) (p<0.001), as shown in (Table 3.3). On the other hand, the study showed the mean ± SD of Mg, Zn, Fe, HDL, in diabetic patients to be lower than in control subjects, Mg (mg/dl) (2.05 ± 0.22) (p<0.01), Fe (µg/dl) (81.91 ± 29.44) (p<0.01), zinc (µg/dl) (75.62 ± 7.54) (p<0.001), HDL (mg/dl) (42.3 ± 8.59) (p<0.01). Other characteristics were not different in a substantial way, cholesterol (mg/dl) (p 0.405), LDL (mg/dl) (p 0.320) (Table 3.3).

Table 3.3. Patients and subjects characteristics according to control, and diabetic patients:

Subjects, characteristics (n=92)	**Frequency Distribution**		**p-value**
	Controls (n=22)	**Diabetic (n=70)**	**(two sides)**
Age (years)*	41.22 ± 7.83	52.12 ±9.09	<0.001
Gender* **	8(36%)	35(50%)	0.398
Male	14(64%)	35(50%)	
Female			
BMI **kg/m^2	27.22±4.55	30.50±4.70	<0.01
Duration of diabetes(years)***	No Diabetic	32(45.7%)	
≤5		38(54.3%)	
>5			
Exercise* **			
Yes	8(36%)	16(23)	0.208
No	14(64%)	54(77%)	
Smoking* **			
Yes	4(18%)	11(16%)	0.785
No	18(82%)	59(84%)	
Glucose (mg/dl)**	93.32±9.18	176.64±59.90	<0.001
HbA1c (%) **	5.25±0.17	8.81±1.71	<0.001
Mg (mg/dl)*	2.22±0.15	2.05±0.22	<0.01
Fe (µg/dl)*	107.95±46.80	81.91±29.44	<0.01
Cu (µg/dl)*	95.04±10.12	100.60±11.47	<0.05
Zn (µg/dl)*	90.04±20.34	75.62±7.54	<0.001
Cholesterol (mg/dl)*	172.13±26.45	178.44±41.61	0.405
Triglyceride (mg/dl)*	128.54±59.45	203.24±80.88	<0.001
HDL (mg/dl)*	50.31±13.35	42.3±8.59	<0.01
LDL (mg/dl)*	108.36±26.71	100.65±39.40	0.320

*mean and S.D for normal, **median and interquartile for non-normal data and ***frequency and percentage were performed in the calculations.

3.3. Trace elements level in patients with T2DM and healthy controls in relations with age group

In our study, there is no relation between trace elements in T2DM and healthy controls with age group, therefore, serum Mg, Zn, Cu, and Fe statistically no significant with age.

Table 3.4. Trace elements level in patients with T2DM and healthy controls in relation with age group:

Biochemical Parameters	**Mean ±SD**			**Mean ±SD**		
	Diabetic (n=70)			Controls (n=22)		
	≤ 40 year (n=4)	> 40 year (n=66)	p-value	≤ 40 year (n=13)	40 > year (n=9)	p-value
Mg	2.15±0.12	2.05±0.22	NS	2.24±0.18	2.18±0.09	NS
Fe	82.25±32.76	81.89±29.50	NS	115.38±48.82	97.22±44.22	NS
Zinc	76.25±7.50	75.59±7.60	NS	90.84±19.71	88.88±22.35	NS
Cu	100.75±9.28	100.59±11.65	NS	95.00±10.89	95.11±9.54	NS
NS: Statistically No Significant						

3.4. Trace elements level in patients with T2DM and healthy controls in relations with BMI group

According to our study, we found there is no significant relation between serum trace elements levels in T2DM patients and control subjects group in relation with BMI, consequently, serum Mg, Zn, Cu, and Fe statistically no significant with BMI. As shown in (Table 3.5).

Table 3.5. Trace elements level in patients with T2DM and healthy controls in relations with BMI group:

Biochemical Parameters	Mean ±SD			
	Diabetic (n=70)			
	< 25 kg/m^2 (2=6)	25-29.9 kg/m^2 (n=30)	≥ 30 kg/m^2 (n=34)	p-value
Mg	2.06±0.12	2.03±0.21	2.07±0.24	NS
Fe	101.33±25.95	75.26±26.05	84.35±31.60	NS
Zinc	79.83±5.30	74.46±7.96	75.91±7.39	NS
Cu	96.83±10.79	100.66±11.77	101.20±11.53	NS
Biochemical Parameters	Mean ±SD			
	Controls (n=22)			
	(n=6)	(n=4)	(n=12)	p-value
Mg	2.28±0.09	2.25±0.12	2.18±0.18	NS
Fe	118±56.53	110.75±64.61	101.91±38.61	NS
Zinc	92.50±23.45	86.00±16.75	90.16±21.33	NS
Cu	90.83±9.10	93.50±7.32	97.66±11.19	NS
NS: Statistically No Significant				

3.5. Trace elements level in patients with T2DM and healthy controls in relations with the gender group

The study showed that the mean ± SD of serum Fe and zinc levels were significantly higher in diabetic patients males group than in the females in the same group with p-value(<0.05) in concerning gender group (Table 3.6). But mean ± SD of serum Mg, Cu, levels were no statistically differences at the diabetic patients' group. On the other hand, we see that the mean ± SD of serum of Mg, Fe, zinc, Cu, statistically no significant differences in study controls subjects in relations with gender group.

Table 3.6. Trace elements level in patients with T2DM and healthy controls in relations with gender group:

Biochemical Parameters	Mean ±SD			Mean ±SD		
	Diabetic (n=70)			Controls (n=22)		
	Male (n=35)	Female (n=35)	p-value	Male (n=8)	Female (n=14)	p-value
Mg	2.09±0.20	2.02±0.23	NS	2.30±0.09	2.17±0.17	NS
Fe	**91.80±27.76**	**72.02±28.0**	**<0.05**	119.37±42.61	101.4±49.34	NS
Zinc	**77.94±6.50**	**73.31±7.89**	**<0.05**	98.62±19.30	85.14±19.89	NS
Cu	99.11±10.32	102.08±12.50	NS	97.62±8.29	93.5±11.05	NS
NS: Statistically No Significant						

3.6. Trace elements level in patients with T2DM in relation with duration group

The study showed that in diabetic patients the level of serum Mg significantly lower in patients who have diabetes mellitus > 5 years than those of ≤ 5 years, with p-value (<0.05). And the other trace elements such as Fe, Cu, and Zn, there is statistically no significant with the group of the duration of diabetes mellitus (Table 3.7).

Table 3.7. Trace elements level in patients with T2DM in relations with Duration group

Biochemical Parameters	Mean ±SD		
	Diabetic (n=70)		
	≤ 5 year (n=32)	> 5 year (n=38)	p-value
Mg	**2.13±0.21**	**1.99±0.20**	**<0.05**
Fe	80.37±31.23	83.21±28.20	NS
Zn	75.09±7.14	76.07±7.94	NS
Cu	98.71±11.22	102.18±11.59	NS
NS: Statistically No Significant			

3.7. Trace elements in patients with T2DM and healthy controls in relations with cholesterol group

Compared to our results, the study showed that there is statistically no significance in trace elements level in T2DM and healthy subjects about the cholesterol group (Table 3.8).

Table 3.8. Trace elements in patients with T2DM and healthy controls in relations with cholesterol group:

Biochemical Parameters	**Mean ±SD**			**Mean ±SD**		
	Diabetic (n=70)			Controls (n=22)		
	Cholesterol level < 200 (n=45)	Cholesterol Level ≥ 200 (n=25)	p-value	Cholesterol level < 200 (n=19)	Cholesterol level ≥ 200 (n=3)	p-value
Mg	2.05±0.21	2.07±0.24	NS	2.22±0.15	2.23±0.20	NS
Fe	80.68±26.93	84.12±33.97	NS	103.78±46.98	134.33±43.87	NS
Zn	75.46±7.27	75.92±8.15	NS	87.84±20.95	104.00±7.21	NS
Cu	99.22±10.31	103.08±13.17	NS	94.78±6.65	96.66±8.12	NS
NS: Statistically No Significant						

3.8. Trace elements in patients with T2DM and healthy controls in relations with triglyceride group

According to our results, the study showed that in the healthy controls group, the level of serum Fe is higher in subjects those have ≥ 150 mg/dl serum triglyceride than those have about < 150 mg/dl triglyceride, with p-value (<0.05), and the other trace elements such as Mg, Zn, and Cu, showed that there is no significant difference in statistical analysis. On the other hand, the study explained that the serum trace elements level in patients with T2DM, a statistically no significant relation to a triglyceride group (Table 3.9).

Table 3.9. Trace elements in patients with T2DM and healthy controls in relations with the triglyceride group:

Biochemical Parameters	**Mean ±SD**			**Mean ±SD**		
	Diabetic (n=70)			Controls (n=22)		
	TG Level < 150 (n=18)	TG Level ≥ 150 (n=52)	p-value	TG Level < 150 (n=16)	TG Level ≥ 150 (n=6)	p-value
Mg	2.04±0.20	2.06±0.22	NS	2.20±0.15	2.28±0.34	NS
Fe	80.22±23.04	82.50±31.53	NS	**95.06±43.16**	**142.32±40.74**	**<0.05**
Zn	75.27±6.73	75.75±7.86	NS	85.43±21.38	102.33±10.74	NS
Cu	96.38±9.45	102.05±11.83	NS	92.93±10.20	100.66±8.14	NS
NS: Statistically No Significant						

3.9. Trace elements in patients with T2DM and healthy controls in relations with HDL group

Related to our results, there is statistically no significant difference showed between trace element levels in patients with T2DM and healthy controls in relations with HDL group (Table 3.10).

Table 3.10. Trace elements in patients with T2DM and healthy controls in relations with HDL group:

Biochemical Parameters	**Mean ±SD**			**Mean ±SD**		
	Diabetic (n=70)			Controls (n=22)		
	Serum HDL level < 40 (n=28)	Serum HDL level ≥ 40 (n=42)	p-value	Serum HDL level < 40 (n=3)	Serum HDL level ≥ 40 (n=19)	p-value
Mg	2.08±0.18	2.03±0.24	NS	2.23±0.05	2.22±0.16	NS
Fe	82.92±33.18	81.23±27.06	NS	96.33±26.53	109.78±49.51	NS
Zn	75.96±8.27	75.40±7.11	NS	90.33±15.69	90.00±21.32	NS
Cu	102.21±11.74	99.52±11.30	NS	100.00±12.52	94.26±13.25	NS
NS: Statistically No Significant						

3.10. Trace elements in patients with T2DM and healthy controls in relations with LDL group

Compared to our results, the study showed that there is no statistically significant in trace elements level in T2DM and healthy subjects concerning the LDL group. (Table 3.11) showed these results.

Table 3.11. Trace elements in patients with T2DM and healthy controls in relations with LDL group:

Biochemical Parameters	**Mean ±SD**			**Mean ±SD**		
	Diabetic (n=70)			Controls (n=22)		
	Serum LDL Level > 130 (n=18)	Serum LDL Level ≤ 130 (n=52)	p-value	Serum LDL Level > 130 (n=5)	Serum LDL Level ≤ 130 (n=17)	p-value
Mg	2.03±0.25	2.06±0.21	NS	2.34±0.08	2.18±0.15	NS
Fe	84.38±29.14	81.05±29.77	NS	138.00±54.27	99.11±42.11	NS
Zn	76.27±7.72	75.40±7.55	NS	99.40±18.91	87.29±20.43	NS
Cu	103.11±12.37	99.73±11.14	NS	99.40±9.78	93.76±10.14	NS
NS: Statistically No Significant						

3.11. Trace element level in the whole study population in relations with a fasting blood glucose level group

Trace elements level in the whole study population concerning fasting blood glucose level group showed that serum of Mg, Fe, and Zn level was considered significantly lower in the subjects those have > 110 mg/dl FBS than those have ≤ 110 mg/dl FBS with p-value (<0.05). Otherwise, the serum Cu was statistically higher in those have > 110 mg/dl FBS than those ≤ 110 mg/dl FBS with p-value (<0.001). (Table 3.12).

Table 3.12. Trac element level in the whole study population in relations with fasting blood glucose level group

Biochemical Parameters	**Mean ±SD**		
	Whole study population (n=92)		
	FBS Level ≤ 110 mg/dl (n=33)	FBS Level > 110 mg/dl (n=59)	p-value
Mg	**2.17±0.18**	**2.05±0.22**	**<0.05**
Fe	**99.21±43.73**	**81.94±29.25**	**<0.05**
Zn	**84.66±18.58**	**75.94±7.69**	**<0.05**
Cu	**93.72±11.0**	**102.37±10.36**	**<0.001**
NS: Statistically No Significant			

3.12. Trace elements levels in the whole study population in relations with HbA1c level group

Trace elements levels in the whole study population in relations with HbA1c level group showed that the serum concentration of Mg and Fe was statistically lower in subjects those have ≥ 6.5 % HbA1c levels, than those of < 6.5 % HbA1c in p-value(<0.05), likewise, serum Zn level also lower in those group of ≥ 6.5 % HbA1c with p-value(<0.01). But serum Cu was considered higher in subjects that have ≥ 6.5 % HbA1c levels, than those of < 6.5 % HbA1c in p-value(<0.001). (Table 3.13).

Table 3.13. Trace elements levels in the whole study population in relations with HbA1c level group:

Biochemical Parameters	Mean ±SD		
	Whole study population (n=92)		
	HbA1c Level < 6.5 (n=30)	HbA1c Level ≥ 6.5 (n=62)	p-value
Mg	**2.18±0.17**	**2.05±0.22**	**<0.05**
Fe	**100.93±46.08**	**81.95±28.13**	**<0.05**
Zn	**85.70±19.30**	**75.87±7.39**	**<0.01**
Cu	**92.80±9.68**	**102.40±10.84**	**<0.001**

NS: Statistically No Significant

3.13. Trace elements in patients with T2DM and healthy controls in relations with the Mg group

During the Mg group concerning trace elements level in patients with Type 2 diabetes mellitus and healthy subjects, the study showed that there is statistically no significant difference found between them (Table 3.14).

Table 3.14. Trace elements in patients with T2DM and healthy controls in relations with Mg group:

Biochemical Parameters	Mean ±SD			Mean ±SD		
	Diabetic (n=70)			Controls (n=22)		
	Serum Mg level ≤ 1.7 (n=12)	Serum Mg level > 1.7 (n=58)	p-value	Serum Mg level ≤ 1.7 (n=2)	Serum Mg level > 1.7 (n=20)	p-value
Fe	82.08±24.57	81.87±30.54	NS	124.50±21.93	106.30±48.62	NS
Zn	76.00±5.65	75.55±7.92	NS	97.50±0.70	89.30±21.22	NS
Cu	105.25±13.88	99.63±10.80	NS	106.00±7.07	93.95±9.83	NS

NS: Statistically No Significant

3.14. Trace elements in patients with T2DM and healthy controls in relations with Fe group

With the Fe group concerning trace elements level in patients with Type 2 diabetes mellitus and healthy controls, the study revealed that there is no statistically significant difference in serum Cu, and Mg, levels in patients with T2DM and healthy controls with Fe group. But the study founded that in the T2DM patients and healthy subjects, the serum Zn is lower in the subjects that have < 50.0 μg/dl Fe level than those of ≥ 50.0 μg/dl Fe level, with p-values(<0.001), (<0.01) respectively (Table 3.15).

Table 3.15. Trace elements in patients with T2DM and healthy controls in relations with Fe group:

	Mean ±SD			**Mean ±SD**		
	Diabetic (n=70)			Controls (n=22)		
Biochemical Parameters	Serum Fe level < 50.0 (n=8)	Serum Fe level ≥ 50.0 (n=62)	p-value	Serum Fe level < 50.0 (n=4)	Serum Fe level ≥ 50.0 (n=18)	p-value
Mg	2.17±0.18	2.04±0.22	NS	2.22±0.09	2.20±0.21	NS
Zn	**65.00±8.43**	**77.00±6.29**	**<0.001**	**63.75±4.27**	**95.88±17.56**	**<0.01**
Cu	102.50±12.14	100.35±11.46	NS	87.75±7.80	96.66±10.02	NS

NS: Statistically No Significant

3.15. Trace elements in patients with T2DM and healthy controls in relations with Zn group

Among the Zn group concerning trace elements level in patients with Type 2 diabetes mellitus and healthy controls, the study revealed that there is no statistically significant difference in Cu, and Mg, level in patients with T2DM and healthy controls with the Zn group. But the study founded that in the T2DM patients and healthy subjects, the serum Fe is lower in the subjects that have < 70.0 μg/dl Zn level than those of ≥ 70.0 μg/dl Zn level, with p-value(<0.001), as shown in the (Table 3.16).

Table 3.16. Trace elements in patients with T2DM and healthy controls in relations with Zn group

Biochemical Parameters	Mean ±SD			Mean ±SD		
	Diabetic (n=70)			Controls (n=22)		
	Serum Zn level < 70.0 (n=13)	Serum Zn level ≥70.0 (n=57)	p-value	Serum Zn level < 70.0 (n=5)	Serum Zn level ≥70.0 (n=17)	p-value
Mg	2.07±0.23	2.05±0.22	NS	2.24±0.08	2.19±0.21	NS
Fe	**42.30±15.47**	**90.94±23.86**	**<0.001**	**42.80±14.49**	**127.11±33.25**	**<0.001**
Cu	102.61±10.52	100.14±11.72	NS	88.20±6.83	97.05±10.19	NS
NS: Statistically No Significant						

3.16. Trace elements in patients with T2DM and healthy controls in relations with Cu group

According to our results, the study showed that in the healthy controls group, the level of serum Fe and Zn is lower in subjects those have < 100 μg/dl serum Cu level than those have about ≥ 100 μg/dl Cu, with p-values(<0.01), (<0.001) respectively, and Mg showed that there is no significant difference in statistical analysis (Table 3.17). On the other hand, the study explained that the serum Mg, Fe, and Zn levels in T2DM patients, a statistically no significant relation to a Cu group.

Table 3.17. Trace elements in patients with T2DM and healthy controls in relations with the Cu group:

Biochemical Parameters	Mean ±SD			Mean ±SD		
	Diabetic (n=70)			Controls (n=22)		
	Serum Cu level < 100 (n=34)	Serum Cu level ≥ 100 (n=36)	p-value	Serum Cu level < 100 (n=14)	Serum Cu level ≥ 100 (n=8)	p-value
Mg	2.06±0.23	2.05±0.21	NS	2.25±0.09	2.13±0.29	NS
Fe	82.58±28.64	81.27±30.56	NS	**85.28±38.77**	**147.62±30.88**	**<0.01**
Zn	76.14±6.58	75.13±8.47	NS	**79.14±15.98**	**109.12±10.57**	**<0.001**
NS: Statistically No Significant						

3.17. Pearson correlation (r) between parameter (correlation analysis)

According to pearson correlation coefficient (r), our results in the whole study population, showed that serum Mg level had a negatively low significant correlation with Cu level (r=-0.240, p= 0.021), and had negatively high significant correlation with each of [Age, FBS, and HbA1c (r=-0.291, p=0.005),(r=-0.285, p=0.006),(r=-0.375, p=0.000) respectively],Table (3.18) shown that. And serum Fe level has positively high significant correlation with Zn (r=0.776, p=0.000), but with FBS and HbA1c we observed a negatively low significant correlation (r=-0.234, p=0.025), (r=-0.260, p=0.012) respectively. About the concentration of Zn elements, we showed that Zn had a negatively high significant correlation with FBS and HbA1c (r=-0.325, p=0.002), (r=-0.365, p=0.000) respectively, conversely we found that Cu had a positively high significant correlation with FBS and HbA1c level with values (r=0.544, p=0.000), (r=-0.594, p=0.000) respectively. (Table 3.18).

Table 3.18. Pearson Correlation (r) between parameter (correlation analysis)

Parameter	Pearson Correlation (r)											
	Mg	**Fe**	**Zn**	**Cu**	**Age**	**BMI**	**CHO**	**TG**	**HDL**	**LDL**	**FBS**	**A1C**
Mg	1											
(r)		0.131	0.155	**-0.240***	**-0.291****	-0.098	0.027	-0.005	0.142	0.037	**-0.285****	**-0.375****
p-value		0.214	0.141	**0.021**	**0.005**	0.350	0.799	-0.960	0.177	0.729	**0.006**	**0.000**
Fe		1										
(r)	0.131		**0.776****	0.085	-0.138	-0.081	-0.002	0.058	-0.113	0.094	**-0.234***	**-0.260***
p-value	0.214		**0.000**	0.422	0.188	0.444	0.982	0.584	0.284	0.374	**0.025**	**0.012**
Zn			1									
(r)	0.155	**0.776****		0.093	-0.167	-0.076	-0.035	-0.059	-0.042	0.056	**-0.325****	**-0.365****
p-value	0.141	**0.000**		0.377	0.111	0.473	0.740	0.575	0.688	0.595	**0.002**	**0.000**
Cu				1								
(r)	**-0.240***	0.085	0.093		0.090	0.161	0.150	0.190	-0.198	0.134	**0.544****	**0.594****
p-value	**0.021**	0.422	0.377		0.394	0.125	0.153	0.070	0.058	0.202	**0.000**	**0.000**

*represent low significant correlation, **represent High significant correlation

T2DM is a big public health concern impacting 200 million people around the world. It is defined by insulin resistance in peripheral tissues and an insulin secretory defect in pancreatic beta cells [34]. Trace elements were recognized as important to optimum safety. The importance of trace elements can not be overemphasized in normal growth, development and overall body metabolism. Nevertheless, the evidence is accumulating that the metabolism of certain trace elements in DM is altered. Many of those trace elements serve as antioxidants and avoid peroxidation of the membrane. The pancreatic beta cells, the cells containing insulin, are susceptible to oxidative stress. This is primarily because their antioxidant intracellular defence mechanisms are poor compared to liver tissues. Thus, oxidative stress is proposed as a possible contributor to diabetes mellitus growth and the complications associated with it, this can not be unconnected to the fact that in diabetic subjects the antioxidant status like antioxidant metal elements may be inadequate, therefore the metabolic value of antioxidant evaluation in diabetic patients is of utmost importance, they can function directly on glucose metabolism in addition to the antioxidant roles of some of those metal elements, the latest research findings revealed a disparity in the amounts of trace elements in T2DM [35]. In our study, we investigate the serum levels of Mg, Fe, Zn, and Cu in patients with T2DM and compared with healthy control subjects.

(Mg) is a cofactor in the process of glucose transporting to the cell membrane and various enzymes in the oxidation of carbohydrates [36]. In this study, we see that the serum Mg level in patients with T2DM was lower than that of the non-diabetic subjects, similar observations were reported by Paul (2004) and Paolisso et al., (1990) and Mamza et al. (2016) [36]. The reasons for reducing Mg in patients with T2DM due to increased urinary loss or impaired Mg absorption; decreased serum Mg may also be attributed to the loss of Mg attributed to osmotic diabetes [36].

(Zn) is a component of many enzymes and has many important linkages to the endocrine system. It is also essential for the normal growth and function of thyroid glands and the metabolism of glucose. In this study, serum Zn levels in Type 2 diabetic subjects were found significantly decreased in than that of the controls group, the findings are consistent with those of Abou Seif, (2004) and Marjani, (2008) and Mamza et al. (2016). The potential explanation of why the serum Zn concentration in diabetic patients decreases is excessive for the urinary secretion of Zn [36].

(Fe) performs an important role as a cofactor for fuel oxidation and the transportation of electrons, yet that, too, is can cause oxidative damage if not properly controlled and occurs in excess tissue, ferritin is an inclusive intracellular protein widely used as a marker for body Fe stores, if the blood comes with low Fe content, ferritin will release Fe, and store excess Fe if the blood and tissues have a high Fe level. Imbalance in diabetes between blood Fe and ferritin is enigmatic, however,

insufficient dietary intake, asymptomatic disease and low absorption levels are usually linked with a decrease in Fe and ferritin. In our study the serum Fe level was found significantly lower in patients with T2DM than healthy controls subjects, that is similar to Wolide et al. (2016) study [37].

(Cu) is well known to play a vitally important role in oxidative stress. Because of their redox chemistry, Cu in its free form is a potent cytotoxic product. It participates readily in reactions by Heiber Weiss and Fenton to produce ROS. A high level of Cu improves the toxic effect of free radicals that rely on metals. Besides, the rise in Cu levels in patients with T2DM may also be due to hyperglycemia, which promotes glycation and triggers the release of Cu ions from protein sites that bind to Cu. The statement of Cu ions into the blood speeds up oxidative stress further [34]. In the present study, we obtained a significant increase in Cu levels in patients with T2DM as compared to controls subjects. Zargar HA et al. showed that Cu levels were significantly elevated in patients with T2DM than in non-diabetic subjects controls. This study is also similar to Kataria et al. (2013) study, that serum Cu level was higher in patients with T2DM than healthy non-diabetic subjects [34].

The mean value of age in diabetic patients was found to be (52.12 ± 9.09 years) which was relatively higher than the mean age of healthy controls subject (41.22 ± 7.83 years) ($p < 0.001$), in our study, there is no relation between trace elements in patients with T2DM and healthy controls subjects, with age group, therefore, serum Mg, Zn, Cu, and Fe statistically no significant with age. (Table 3.4). This statistic is similar to each of Hami, Ibrahim and Ahmed et al. 2016 study, and Hasan, Ahjel, Odhar, Hussein, and Alhaideri et al. 2019 study, and Wolide et al. 2016 study [37, 38, 39].

It is common scientific understanding that there is a clear connection between patients with T2DM and increased BMI and FBS, having an average overweight BMI indicates a possible interplay of genetic factor, lack of exercise and inactive lifestyle [40]. Our study showed that the mean±SD of BMI in diabetic patients was greater than in the control subjects, (30.50 ± 4.70), (27.22 ± 4.55) (kg/m^2) respectively, in value ($p<0.01$), according to our study, we found there is no statistically significant relation between serum trace elements levels in patients with T2DM, and healthy controls group, in relations with BMI, (Table 3.5), several previous studies are similar to our study like Wolide et al. 2016 study [37].

The study considered 70 diabetic patients 35 males and 35 females and it showed that the mean ± SD of serum Fe and Zn levels was significantly higher in diabetic patients males group than in the females in the same group with p-value(<0.05) in concerning gender group, (Table 3.6). The study of Lamsal, Sharma, and Baral et al. 2014 showed that the serum of Fe is lower in females diabetic patients than the male that is similar to our study, also similar observation was reported by Saharia

and Goswami et al. 2013 who showed serum Zn was significantly lower in females diabetic patients than the males' diabetic patients [41, 42].

The mean ± SD of glucose in diabetic patients is higher than healthy controls (176.64 ± 59.90) (mg/dl), ($p<0.001$) and trace elements level in the whole study population concerning fasting blood glucose level group showed that serum of Mg, Fe, and Zn level was considered significantly lower in the subjects those have > 110 mg/dl FBS than those have ≤ 110 mg/dl FBS with p-value(<0.05). Otherwise, the serum Cu was statistically higher in those have > 110 mg/dl FBS than those ≤ 110 mg/dl FBS with p-value (<0.001), (Table 3.12). The observation was similar to many studies such as Mamza et al. (2016) and Wolide et al. 2016 study [36,37]. But there is other studies have different results than of our results example of that Masood, Baloch, Ghoriet al. 2009, and Hasan, Ahjel, Odhar, Hussein, and Alhaideri et al. 2019 and Basaki, Saeb, Shamsaeiet al. 2012 [39][43][3]. The higher levels of fasting blood glucose seen in diabetic patients result from insulin deficiency or diabetes mellitus associated insulin resistance [35].

The mean ± SD of HbA1c (%) in diabetic patients is higher than healthy controls (8.81 ± 1.71) ($p<0.001$), trace element level in the whole study population in relations with HbA1c level group showed that the serum concentration of Mg and Fe was statistically lower in subjects those have ≥ 6.5 % HbA1c levels, than those of < 6.5 % HbA1c in p-value(<0.05), likewise, serum Zn level also lower in those group of ≥ 6.5 % HbA1c with p-value(<0.01). But serum Cu was considered higher in subjects that have ≥ 6.5 % HbA1c levels, than those of < 6.5 % HbA1c in p-value(<0.001), (Table 3.13). This close finding to other studies, such as Mamza et al. (2016), and Wolide et al. 2016 study [36][37], yet other studies have findings different from our results, an example of that Masood, Baloch, Ghoriet al. 2009, and Hasan, Ahjel, Odhar, Hussein, and Alhaideri et al. 2019 and Basaki, Saeb, Shamsaei et al. 2012 [39, 43, 3]. The present study presents a significant indication that altered metabolism of Mg, Cu, Zn, and Fe is closely correlated with increased HbA1c levels. These associations may represent a risk factor for diabetic complications to develop. Our findings suggest that potential changes in the metabolism of these metals, in particular their association with long-term hyperglycemia, need to be taken into account [35].

In this current study, the mean of the patients that have diabetes mellitus during ≤ 5 years was (32), (45.7 %), and the (38), (54.3 %) in the case that has in > 5 years. Our study showed that in diabetic patients the level of serum Mg significantly lower in patients who have diabetes mellitus > 5 years than those of ≤ 5 years, with p-value (<0.05). These result similar to Devrajani et al. 2015 stud [44].

According to the Pearson correlation coefficient (r), our results in the whole study population showed that serum Mg level had a negatively low significant correlation with Cu level and also had a negatively high significant correlation with each of Age, FBS, and HbA1c, these results supported by Wolide et al. 2016 study [37]. And serum Fe level has a positively high significant correlation with Zn this is similar to Atari-hajipirloo, Valizadeh, et al. 2016 study [45]. But in the correlation of serum Fe with FBS and HbA1c, we observed a negatively low significant correlation the same results we founded in the Wolide et al. 2016 study [37]. About the concentration of Zn element, we showed that Zn had a negatively high significant correlation with FBS and HbA1c as similar to the study of Farid and Abulfaraj et al. 2013 [35]. Conversely, we found that Cu had a positively high significant correlation with FBS and HbA1c level study of Farid and Abulfaraj et al. 2013 and Wolide et al. 2016 supported our results [35, 37]. (Table 3.18).

4. CONCLUSIONS

In conclusion, the current study shows a reduction in the serum of Fe, Zn, and Mg levels, and an increase of serum Cu levels in patients with T2DM (cases group) than that in healthy non-diabetic patients (controls group). The present study provides significant evidence confirming that altered metabolism of Fe, Zn, Mg, and Cu is strongly linked with the increased levels of FBS and HbA1c. Such connections may be a risk factor for developing diabetic complications. We think that poor glycemic regulation, poor adherence to insulin and oral hypoglycemic agents, high urinary clearance levels, asymptomatic disease, oxidative stress and other factors related to safety and safety may contribute to the current disrupted trace elements in patients with T2DM. So, following the possible preventable strategies for example lifestyle modification and good adherence to medications may reduce the complications of diabetes and trace elements in Type 2 diabetes mellitus patients.

REFERENCES

[1] K. Siddiqui, N. Bawazeer, and S. Scaria Joy, (2014)., "Variation in macro and trace elements in progression of type 2 diabetes," *Sci. World J.*, vol. no. Figure 1, 2014, doi: 10.1155/2014/461591.

[2] C. R. Flores, M. P. Puga, K. Wrobel, M. E. Garay Sevilla, and K. Wrobel, (2011)., "Trace elements status in diabetes mellitus type 2: Possible role of the interaction between molybdenum and copper in the progress of typical complications," *Diabetes Res. Clin. Pract.*, vol. 91, no. 3, pp. 333–341, doi: 10.1016/j.diabres.2010.12.014.

[3] M. Basaki, M. Saeb, S. Nazifi, and H. A. Shamsaei, (2012)., "Zinc, copper, iron, and chromium concentrations in young patients with type 2 diabetes mellitus," *Biol. Trace Elem. Res.*, vol. 148, no. 2, pp. 161–164, doi: 10.1007/s12011-012-9360-6.

[4] S. N. Rajpathak, J. P. Crandall, J. Wylie-Rosett, G. C. Kabat, (2009).,T. E. Rohan, and F. B. Hu, "The role of iron in type 2 diabetes in humans," *Biochim. Biophys. Acta - Gen. Subj.*, vol. 1790, no. 7, pp. 671–681, doi: 10.1016/j.bbagen.2008.04.005.

[5] Y. Z. Guangcui Xu, (2015)., "Type 2 Diabetes Mellitus- Disease, Diagnosis and Treatment," *J. Diabetes Metab.*, vol. 06, no. 05, doi: 10.4172/2155-6156.1000533.

[6] Y. Zheng, S. H. Ley, and F. B. Hu, (2017)., "Global aetiology and epidemiology of type 2 diabetes mellitus and its complications," *Nat. Rev. Endocrinol.*, vol. 14, no. 2, pp. 88–98, 2018, doi: 10.1038/nrendo.151.

[7] N. Salam, M. Fareed, A. T. Khoja, M. Abdulrahman Mahmoud, and M. Ahamed, (2017)., "Life Style Related Risk Factors of Type 2 Diabetes Mellitus and Its Increased Prevalence in Saudi Arabia: A Brief Review Utilization of Clinical Preventive Services Among Saudi Older Adults View project Life Style Related Risk Factors of Type 2 Diabetes M," *www.ijmrhs.com Int. J. Med. Res. Heal. Sci.*, vol. 6, no. 3, pp. 125–132,

[8] J. Wang and K. Pantopoulos, (2011)., "Regulation of cellular iron metabolism," *Biochem. J.*, vol. 434, no. 3, pp. 365–381, doi: 10.1042/BJ20101825.

[9] Clinical Nutrition Services Department UWH, "Iron in Your Diet," pp. 1–3, (2009).

[10] N. I. of Health, (2016)., "Iron Fact Sheet for Consumers Iron," *U.S Dep. Heal. Hum. Serv.*,

[11] I. Protein and P. Subunits, (2007)., “Iron Use and Storage in the Body : Ferritin : The Iron-Storage Protein,” pp. 1–12,

[12] G. J. Anderson and D. M. Frazer, (2017)., “Current understanding of iron homeostasis,” vol. 106, pp. 1559–1566, doi: 10.3945/ajcn.117.155804.

[13] Y. Kohgo, K. Ikuta, T. Ohtake, Y. Torimoto, and J. Kato, (2008)., “Body iron metabolism and pathophysiology of iron overload,” *Int. J. Hematol.*, vol. 88, no. 1, pp. 7–15, doi: 10.1007/s12185-008-0120-5.

[14] G. Targher, M. Franchini, M. Montagnana, and G. Lippi, (2007)., “The role of iron in diabetes and its complications: Reponse to Swaminathan et al. [5],” *Diabetes Care*, vol. 30, no. 12, 2007, doi: 10.2337/dc07-1633.

[15] K. Kostov, (2019)., “Effects of magnesium deficiency on mechanisms of insulin resistance in type 2 diabetes: Focusing on the processes of insulin secretion and signaling,” *Int. J. Mol. Sci.*, vol. 20, no. 6, doi: 10.3390/ijms20061351.

[16] U. Gröber, J. Schmidt, and K. Kisters, (2015)., “Magnesium in prevention and therapy,” *Nutrients*, vol. 7, no. 9, pp. 8199–8226, doi: 10.3390/nu7095388.

[17] W. J. Fawcett, E. J. Haxby, and D. A. Male, (1999)., “Magnesium: Physiology and pharmacology,” *Br. J. Anaesth.*, vol. 83, no. 2, pp. 302–320, doi: 10.1093/bja/83.2.302.

[18] “Chapter 14,” *Acts Apostles Passages Transl.*, pp. 223–233, (2014), doi: 10.5040/9781472539564.ch-014.

[19] G. Schwarz and B. Aa, (2013)., *Cobalt in human health and disease*, vol. 13.

[20] M. Barbagallo and L. J. Dominguez, “Magnesium and Health Internal medicine : Open Access,” pp. 1–2.

[21] T. Kambe, T. Tsuji, A. Hashimoto, and N. Itsumura, (2015)., “The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism,” *Physiol. Rev.*, vol. 95, no. 3, pp. 749–784, doi: 10.1152/physrev.00035.2014.

[22] K. Kaur, R. Gupta, S. A. Saraf, and S. K. Saraf, (2014)., “Zinc: The metal of life,” *Compr. Rev. Food Sci. Food Saf.*, vol. 13, no. 4, pp. 358–376, doi: 10.1111/1541-4337.12067.

[23] T. A. Strand *et al.*, (2018)., “Assessment of Zinc Intake in Relation to Tolerable Upper Intake Levels,” *Eur. J. Nutr. Food Saf.*, vol. 8, no. 4, pp. 243–244, doi: 10.9734/ejnfs/2018/43860.

[24] J. Deshpande, M. Joshi, and P. Giri, (2013)., "Zinc: The trace element of major importance in human nutrition and health," *Int. J. Med. Sci. Public Heal.*, vol. 2, no. 1, p. 1, 2013, doi: 10.5455/ijmsph. 2.1-6.

[25] V. J. Temple and A. Masta, (2004)., "Zinc in human health.," *P. N. G. Med. J.*, vol. 47, no. 3–4, pp. 146–158, doi: 10.9790/0853-13721823.

[26] A. Toma, E. Makonnen, and G. Yimer, (2013)., "Role of zinc in diabetes mellitus, oxidative stress and other human healthy: a review article," *Am. J. Res. Commun.*, vol. 1, no. 11, pp. 411–426,

[27] H. Tapiero, D. M. Townsend, and K. D. Tew, (2003)., "Trace elements in human physiology and pathology. Copper," *Biomed. Pharmacother.*, vol. 57, no. 9, pp. 386–398, doi: 10.1016/S0753-3322(03)00012-X.

[28] M. Bost, S. Houdart, M. Oberli, E. Kalonji, J. F. Huneau, and I. Margaritis, (2016)., "Dietary copper and human health: Current evidence and unresolved issues," *J. Trace Elem. Med. Biol.*, vol. 35, pp. 107–115, doi: 10.1016/j.jtemb.2016.02.006.

[29] C. A. Copper Development Association, (2018)., "Copper Essential for Human Health," pp. 1–7,

[30] M. Olivares and R. Uauy, (1996)., "Copper as an essential nutrient," *Am. J. Clin. Nutr.*, vol. 63, no. 5, doi: 10.1093/ajcn/63.5.791.

[31] J. Osredkar, (2011)., "Copper and Zinc, Biological Role and Significance of Copper/Zinc Imbalance," *J. Clin. Toxicol.*, vol. s3, no. 01, pp. 1–18, doi: 10.4172/2161-0495.s3-001.

[32] N. S. Ahmad, F. Islahudin, and T. Paraidathathu, (2014)., "Factors associated with good glycemic control among patients with type 2 diabetes mellitus," *J. Diabetes Investig.*, vol. 5, no. 5, pp. 563–569, doi: 10.1111/jdi.12175.

[33] R. Range, (2016)., "ABIM Laboratory Reference Ranges -- July 2014," vol. 6, no. July, pp. 3–10,

[34] R. Kataria, G. Singh, A. Gupta, S. Jalhan, and A. Jindal, (2013)., "Academic Sciences Asian Journal of Pharmaceutical and Clinical Research," *Asian J. Pharm. Clin. Res.*, vol. 6, no. April 2016, pp. 5–7,

[35] S. M. Farid and T. G. Abulfaraj, (2013)., "Trace Mineral Status Related to Levels of Glycated Hemoglobin of Type 2 Diabetic Subjects in Jeddah , Saudi Arabia," *Med. J. Islam. World Acad. Sci.*, vol. 21, no. 2, pp. 47–56, doi: 10.12816/0001489.

[36] Y. P. Mamza, Z. B. Abdullahi, R. M. Gali, D. Mshelia, R. Y. Genesis, and S. A. Habu, (2016)., "Status of Serum Zinc and Magnesium among Type 2 Diabetic Subjects in Maiduguri," *IOSR J. Dent. Med. Sci.*, vol. 15, no. 07, pp. 66–70, doi: 10.9790/0853-150716670.

[37] A. D. Wolide, B. Zawdie, T. Alemayehu, and S. Tadesse, (2016)., "Evaluation of serum ferritin and some metal elements in type 2 diabetes mellitus patients: Comparative cross-sectional study," *Diabetes, Metab. Syndr. Obes. Targets Ther.*, vol. 9, pp. 417–424, doi: 10.2147/DMSO.S120326.

[38] M. Hami, A. Ibrahim, and M. Ahmed, (2016)., "The Status of Serum Zinc, Magnesium and Calcium in Type 2 Diabetic Patients and Their Correlation with Renal Function," *Sci. J. Univ. Zakho*, vol. 4, no. 2, pp. 208–212, doi: 10.25271/2016.4.2.84.

[39] M. A. Hasan, S. W. Ahjel, H. A. Odhar, R. M. Hussein, and M. R. A. Alhaideri, (2019)., "Clarifying the relationship of serum levels of iron and copper with type 2 diabetes mellitus," *Indian J. Forensic Med. Toxicol.*, vol. 13, no. 4, pp. 885–888, doi: 10.5958/0973-9130.2019.00408.0.

[40] N. Agrawal, M. K. Agrawal, T. Kumari, and S. Kumar, (2017)., "Correlation between Body Mass Index and Blood Glucose Levels in Jharkhand Population," vol. 4, no. 8, pp. 1633–1636,

[41] M. Lamsal, S. K. Sharma, and N. Baral, (2014)., "Status of iron , oxidant and antioxidants in chronic type 2 diabetes mellitus patients," no. September,

[42] G. K. Saharia and R. K. Goswami, "Evaluation of Serum Zinc Status and Glycated Hemoglobin of Type 2 Diabetes Mellitus Patients in a Tertiary Care Hospital of Assam," no. 1, pp. 30–34, 2013, doi: 10.4103/0974-2727.115923.

[43] N. Masood, G. H. Baloch, R. A. Ghori, I. A. Memon, M. A. Memon, and M. S. Memon, (2009)., "Serum zinc and magnesium in type-2 diabetic patients," *J. Coll. Physicians Surg. Pakistan*, vol. 19, no. 8, pp. 483–486,

[44] B. R. Devrajani and H. Sciences, (2015)., "Hypomagnesemia in Patients with Diabetes mellitus," no. February,

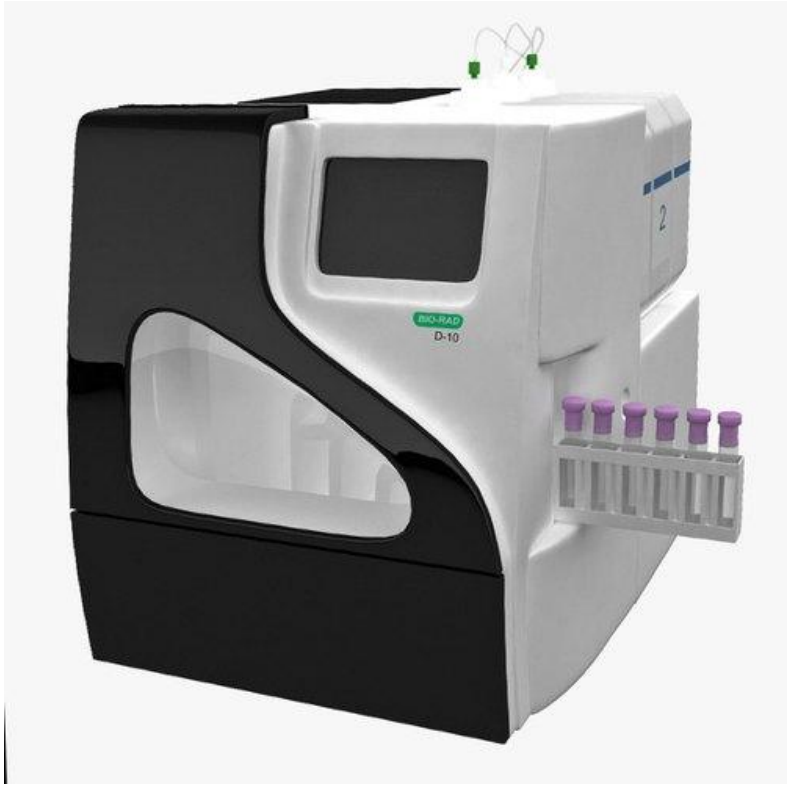
[45] S. Atari-hajipirloo, N. Valizadeh, Y. Rasmi, and F. Kheradmand, (2016)., “Altered Concentrations of Copper , Zinc , and Iron are Associated With Increased Levels of Glycated Hemoglobin in Patients With Type 2 Diabetes Mellitus and Their First-Degree Relatives,” vol. 14, no. 2, pp. 10–16, doi: 10.5812/ijem.33273.Research.

APPENDICES

APPENDIX (1) COBAS C311 AUTOANALYZER

APPENDIX (2) BIOLIS 24IAUTOANALYZER

APPENDIX (3) HPLC D10 INSTRUMENT BIO-RAD



APPENDIX (4) RESEARCH QUESTIONNAIRE

1) Patients name: ……
2) Patients ID:……
3) Age: …….
4) Job: ……..
5) Address: ……..
6) Tel.: …….

Diseases History

• Chronic kidney diseases:	Yes	No
• Chronic liver diseases:	Yes	No
• General inflammation:	Yes	No
• Smoker	Yes	No
• Types of diabetes therapy		
• Duration of daisies	≤5 Years	>5 Years
• Physical activity	Yes	No
• Taking medications including:		
-Folic acid	Yes	No
-Multi-Vitamins	Yes	No
-Insulin	Yes	No

Physical examinations

1. Width circumferences (WC)…… ( )
2. BMI……...(kg/m^2)

Weight:.....(kg) Height…….(cm)

APPENDIX (5): FORM (B) APPROVAL OF RESEARCH ETHICS COMMITTEE

Kurdistan Regional Government
Ministry of Health
Duhok Directorate General of Health
Directorate of Planning
Scientific Research Division

Form B: Approval of Research Ethics Committee

Date: 10th September, 2019

Reference number: 10092019-6

Research title: Evaluation of serum trace elements in patients with type 2 Diabetes Mellitus.

Researcher name(s): Sipan Askander Rasheed.

Type of approval: Conditional, taking into consideration the following mandates:

1) Obtaining the approval of Azadi teaching hospital.
2) Filling the Consent Form (Form C) for all the participants in the study and returning them to Research Ethics Committee at Duhok Directorate General of Health after completion of the study.
3) Grantee of this letter is not given the right to publish the results of the study until returning Form C (Consent Form). Upon returning these forms, the researcher(s) will be provided by unconditional letter from Research Ethics Committee to publish the results
4) The participants don't bear the responsibility for paying for any procedure.
5) The participants have the right to withdraw from the study.
6) Confidentiality of the data should be secured.
7) Notifying the participant about the results of the procedure performed.
8) Any change in the methods of the study needs prior approval of Research Ethics Committee.

Dr. Karwan Hassan Sanaan
Member

Dr. Nazik Abdulraheem Abdulkareem
Chairman

Dr. Hashim Dawood Musa
Member

Dr. KaramSaeed Sulaiman.
Member

DIRECTORATE GENERAL OF HEALTH - DUHOK
DGoH DUHOK
RESEARCH ETHICS COMMITTEE

Dr. Dilovan AbdulMajed
Member

Address:
Room 4, Floor 2,
Duhok Directorate General of Health
Duhok, Kurdistan Region, Iraq
Postcode: 42001

Contact:
Email: scientific.research@duhokhealth.org
Tel.: +964(0)62 724 2786
Web: www.duhokhealth.org

APPENDIX (6): FORM (B) APPROVAL OF RESEARCH ETHICS COMMITTEE

Kurdistan Regional Government
Ministry of Health
Duhok Directorate General of Health
Directorate of Planning
Scientific Research Division

Form B: Approval of Research Ethics Committee

Date: 31 May, 2020

Reference number: 10092019-6.

Research title: Evaluation of serum trace elements in patients with type 2 Diabetes Mellitus.

Researcher name(s): Sipan Askander Rasheed.

Type of approval: Conditional.

The forms submitted by the researcher meet the ethical requirements mandated by Research Ethics Committee. Grantee of this letter is given the right to publish the results of the study.

Dr. Nazik A. Abdulkarim
Research Ethics Committee
Duhok Directorate General of Health
Duhok, Iraq

DIRECTORATE GENERAL OF HEALTH – DUHOK
DGoH DUHOK
RESEARCH ETHICS COMMITTEE

Address: Room 1, Floor 2,
Duhok Directorate General of Health
Duhok, Kurdistan Region, Iraq

CURRICULUM VITAE

Sipan Askandar Rasheed ALMIZORI

Personal Information

Birth of Place : Iraq
Birth of Date : 25/01/1989
Nationality : Iraqi
Address : Duhok
E-mail : sipan.askander89@gmail.com
Languages : Arabic, Kurdish, English

Education

Bachelor : Bachelor of Science (BSc.). Chemistry. University of Duhok (2010-2011)
High School : Kawa, Duhok City, the year 2007

Work Experience

2013 – 2018 : Works as chemical analyzer in Duhok Emergency teaching hospital