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Title

Evaluation of Serum Trace Elements among Sudanese Diabetic Patients in River Nile State

By

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بسم الله الرحمن الرحيم

قال تعالي:

(قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

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Dedication

- **To my family**
- **To my teachers**
- **To my colleagues**
- **To my friends**

I dedicate this work with my best wishes to all

And also To the soul of Dr. Omer Abass Elmehena

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Abstract….

Background: Diabetes mellitus is a chronic disorder that is associated with the imbalance of trace elements which are involved in many functions especially enzyme activities and this imbalance can create some diabetic complications.

There is accumulating evidence that the metabolism of several trace elements is altered in diabetes mellitus and that these nutrients might have specific roles in the pathogenesis and progress of this disease

Aim: The aim of our study was to compare the level of trace elements {copper (*Cu*), iron (*Fe*), magnesium (*Mg*) and zinc (*Zn*)} in serum samples of patients diagnosed with diabetes mellitus (n = 182), with those nondiabetic control subjects (n = 60) with different ages of both genders, and measure its association with glycemic control and other diabetic complications.

Research Design and Methods: This is case-control study conducted during January 2015 to June 2017.

The element concentrations were measured by atomic absorption spectrophotometer. Also Glucose, creatinine, cholesterol, triglycerides, *(ACR)* levels were measured by spectrophotometer and *HA1c* by i-chroma (immunoflorescence based technique) also Body mass index was calculated from each person's (weight and height) and also measured waist lines and blood pressure.

Venous blood sample (separated to serum and whole blood in *EDTA*) were obtained from diabetic patients and control group for these parameters. The data were analyzed using the SPSS software version (11.5).

Results: The study showed The Serum (Cu), (Zn), (Mg) and (Fe) were lower in case group than control group and there was negative correlation between (Cu) and *HbA1c* and no effect of glucose control to (Zn), (Mg) and (Fe).

The (Cu) and Fe were *lower in type II* of diabetic patients than *type I DM* while Mg was lower in *type I DM* than *type II DM*

The level of (Cu) was reduced with increase duration of DM and low level of Fe found in group of higher duration (more than 10 years).

Conclusions: The Serum (Cu), (Zn), (Mg) and (Fe) were lower in case group than control group and there was a negative correlation between (Cu) and *HbA1c*.

المستخلص

الخلفية: مرض السكرمن الامراض المزمنة ويترافق مع اختلال فى تراكيز العناصر الشحيحة فى جسم الانسان والتى لها اهمية لكثير من الوظائف خاصة عمل الانزيمات. التغير فى تركيز هذه العناصر قد يؤدى الى مضاعفات عند مرضى السكرى. وهناك بعض الدلائل التى تشير الى ان اختلال عمليات الاستقلاب فى هذه العناصر يؤثر فى امراضية وتطور مرض السكرى.

الهدف: الهدف من هذه الدراسة هومقارنة تركيز العناصر الشحيحة (الزنك، النحاس، الماغنيزيوم والحديد) بين مرضى السكرى السودانين (182 مريض) مع اصحاء كعينة ضابطة (60 شخص) بمختلف الاعمار من الجنسين.

نوع الدراسة والطريقة: اجريت هذه الدراسة الوصفية فى الفترة بين يناير 2015 الى يونيو 2017 .

تم قياس العناصر الشحيحة بجهاز الامتصاص الزرى، كذلك تم قياس الجلكوز، الكرياتنين، الكوليستيرول و الدهون الثلاثي عن طريق جهاز الاسبكتروفوتوميتر، وايضا تم قياس اسكر الدم التراكمى عن طريق جهاز الايكروما.

كذلك تم قياس الطول والوزن ومن ثم قياس معدل كتلة الجسم وكذلك قياس محيط الخصر وضغط الدم. ومن ثم تحليل النتائج.

النتائج: توصلت الدراسة الى انة يوجد انخفتض فى تراكيز العناصر الشحيحة (الزنك، النحاس، الماغنيزيوم والحديد) عند مرضى السكرى مقارنة بالمجموعة الضابطة

وهناك ترافق عكسى بين مستوى النحاس ومعدل السكر التراكمى ولايوجد تاثير فى بقية العناصر (الزنك، الماغنيزيوم والحديد).

كذلك وجدت الدراسة الى ان مستوى النحاس يزيد عند مرضى السكرى من النوع الاول بينما يقل مستو الماغنيزيوم عند نفس النوع مقارنة بالنوع الثانى.

كذلك يقل مستوى النحاس بزيادة زمن المرض بينما نجد ان الحديد يزداد عند الذين يكون عندهم المرض لفترة اكثر من عشر سنوات.

الخلاصة: يوجد انخفتض فى تراكيز العناصر الشحيحة (الزنك، النحاس، الماغنيزيوم والحديد) عند مرضى السكرى مقارنة بالمجموعة الضابطة و هناك ترافق عكسى بين مستوى النحاس ومعدل السكر التراكمى.

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Abbreviations

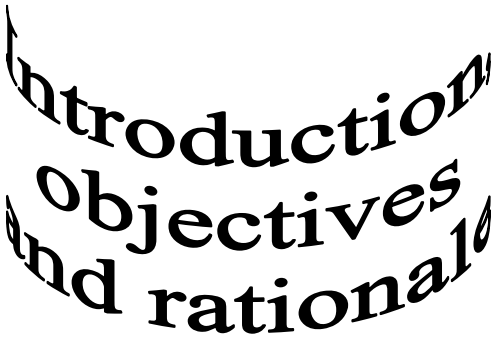
1.	AAS	Atomic absorption spectrometry
2.	ACR	Albumin creatinine ratio
3.	ADA	American diabetic association
4.	As	Arsenic
5.	BMI	Body mass index
6.	C3	Complement factor 3
7.	Cd	Cadmium
8.	CNS	Central nerves system
9.	Co	Cobalt
10.	Cr	Chromium
11.	CRP	C reactive protein
12.	Cu	Copper
13.	DM	Diabetes mellitus
14.	Fe	Iron
15.	GH	Growth hormone
16.	GIT	Gastro intestinal tract
17.	GSK3	Glycogen synthase kinase 3
18.	H_2O_2	Hydrogen peroxide
19.	Hb	Hemoglobin
20.	HbA1c	Hemoglobin A_{1C}
21.	HDL	High density lipoproteins
22.	HLA	Human leukocyte antigen
23.	HPLC	High performance liquid chromatography
24.	LDL	Low density lipoproteins
25.	Li	Lithium
26.	Mg	Magnesium
27.	Mn	Manganese
28.	Mo	Molybdenum
29.	NEFA	Non esterified fatty acid
30.	Ni	Nickel
31.	OGTT	Oral glucose tolerance test
32.	Pb	Lead
33.	ROS	Reactive oxygen species

34.	Se	Selenium
35.	SOD	Superoxide dismutase
36.	T3	Triiodothyronine
37.	T4	Tetraiodothyronine or thyroxin
38.	TNFα	Tumor necrosis factor alpha
39.	TSH	Thyroid stimulating hormone
40.	Type 1 DM	Type one diabetes mellitus
41.	Type II DM	Type tow diabetes mellitus
42.	UK	United kingdom
43.	Va	Vanadium
44.	VLDLs	Very low density lipoprotein
45.	ZAG	Zn-α2-glycoprotein
46.	Zn	Zinc

Unit's abbreviations

1.	g	gram
2.	mg	milligram
3.	µg	microgram
4.	nm	nanometer
5.	µmol	micromol
6.	L	litter
7.	dl	deciliter
8.	ml	milliliter
9.	µl	microlitter
10.	Yrs	years
11.	hr	hour
12.	min	minute

Chapter One



1.1Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. *DM* is associated with abnormalities in carbohydrates, fats and protein metabolism.(1)

Oxidative stress is one of major risk factors in diabetes progression, Lifestyle factors, such as obesity and unhealthy eating habits, as well as increased age disturb redox balance in the body and influence insulin sensitivity. Hyperglycemia exacerbates oxidative stress and leads to the development of microvascular (*retinopathy, nephropathy, neuropathy)* and macrovascular complications (*cardiovascular disease*). Complications of *DM* do not only significantly deteriorate diabetic patients' health but also increase the costs of healthcare. (2)

Many trace elements, among which metals are indispensable for proper functioning of a myriad of biochemical reactions, more particularly as enzyme cofactors. This is particularly true for the vast set of processes involved in regulation of glucose homestasis, being it in glucose metabolism itself or in hormonal control, specially insulin. (3)

Trace elements are inorganic constituents present at very low concentrations in bodily fluids. The biological effects of deficiency disease define the essential trace elements; an element is considered essential when the signs and symptoms induced by a deficient diet are uniquely reversed by an adequate supply of the particular trace element under investigation. The functions of trace elements include being a structural component of a vitamin c and being a co-factor in metalloenzymes, such as glutathione peroxidase *Zinc (Zn) and copper (Cu)*, catalytic components of numerous enzymes, are also structural components of other important proteins. Nutrient status, including the bioavailability and concentration of trace elements, is known to be influenced by many physiological and diet factors, such as soil,

geographical location, food preparation, pollution, body composition, and ethnicity. During pregnancy, both physiological variables and diet influence the availability of trace elements for digestion, absorption, and utilization. [4]

Trace elements, such as (*Zn)* and (*Cu)* may play a pivotal role in the pathogenesis of diabetes and diabetic complications by mediating oxidative stress. (*Zn)* deficiency is associated with an increased risk of type II *DM* and cardiovascular disease. [5]

The metabolism of several trace elements has been reported to alter in *DM* and these elements might have specific roles in the pathogenesis and progress of this disease. [6]

Appropriate nutrition of all the metabolically active cells and tissues is essential for preserving health of the human body as a whole. Micronutrients, including trace elements, vitamins, and antioxidants, play a vital role in continuously occurring regenerative processes, coping with ongoing oxidative stress in the body tissues, and sustaining ample immunity against pathogens. The manifestations of undernutrition as well overnutrition of micronutrients on the oral health are vast and can result in defects of the dental hard tissues as well as oral mucosa. [7]

The word “trace elements” is used for elements existing in natural and perturbed environments in small amounts, with excess bioavailability having a toxic effect on the living organism. Trace elements are chemical micronutrients which are required rather in minute quantity but play a vital role in maintaining integrity of various physiological and metabolic processes occurring within living tissues. The deficiency of any of the trace elements may be apparent as a combination of various clinical manifestations rather than a specific presentation as each trace element is related to many enzyme systems. [7]

Healthy nutritional habits with regular intake of essential vitamins and minerals are of immense significance to general as well as oral health. As there had been limited

knowledge among the oral physicians regarding significance of trace elements in human nutrition, the current review focuses on the role of those essential trace elements which have a proven role in maintaining oral health and their implications in various oral diseases and disorders. [7]

1.2. Rationale

There is high prevalence of *DM* and glucose intolerance in the urban population of the River Nile State. [8] and High prevalence of uncontrolled *DM* (85%) is noted in Sudanese individuals with type II *DM*. [9]

Also there is a limited local data about trace elements in Sudanese diabetic patients. This study aims to provide more data about diabetes in Sudan which will help authorities to evaluate the problem more objectively and implement appropriate measures to reduce complications, morbidity and mortality of diabetes in Sudan.

Diabetes mellitus is supposed to be associated with fluctuations in the plasma levels of several trace elements. There is accumulating evidence that the metabolism of several trace elements is altered in patients with diabetes mellitus and that these nutrients might have specific roles in the pathogenesis and progression of this disorder.

Many trace elements are indispensable for proper functioning of a myriad of biochemical reactions, more particularly as enzyme cofactors. This is particularly true for the vast set of processes involved in regulation of glucose homeostasis, being it in glucose metabolism itself or in hormonal control, especially insulin. (3)

In 2000, according to the World Health Organization, at least (171) million people worldwide suffer from diabetes, or (2.8%) of the population. Its incidence is increasing rapidly, and it is estimated that by the (year 2030), this number will almost double. *DM* occurs throughout the world, but is more common (specially *type II DM*) in the more developed countries.

In Sudan, also diabetes continues to be a particularly serious medical and social problem like anywhere else in the world. Moreover, children are falling victims to it increasingly often. A lot can be done to prevent or slow down diabetes problems

such as follow the healthy eating, take your medicines as directed and check your blood glucose regularly with record.

1.3 Objectives:

1.3.1 General objective:

The aim of the study was to measure the level of some trace elements in diabetic patients and compare it with healthy people as control group**.**

1.3.2 Specific objectives:

- To correlate HbA1c, BMI, waist circumference, ACR, plasma lipids and serum creatinine with trace elements levels in *DM*.
- To evaluate the effects of types of *DM* on trace elements level.
- To determine the effect of duration of *DM* on HbA1c level.
- To correlate between microalbuminuria, BMI and waist circumference with HbA1c level in *DM*.
- To correlate the occurrence of diabetic complications with the level trace elements.

Chapter two

Literature review

Literature review

2.1 Diabetes Mellitus:

2.1.1 Definition:

DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. *DM* is associated with abnormalities in carbohydrates, fats and protein metabolism. (1)

The disorders of diabetes differ in their etiology and symptoms and in the consequences of disease. It is a serious public health threat and economic burden on healthcare funds. specific therapeutic intervention may reduce the serious consequences of diabetes. To aid the physician in choosing appropriate therapy, the laboratory plays a role in diagnosis of the disease, identification of the type of the disorder, and assessment of progression of the tissue damage. Insulin replacement, diet management, and exercise have been shown to reduce the consequences of *type 1 DM*. *Type II DM* is best controlled by weight loss, diet management, and drug therapy, such as sulphonylureas, benzoic acid analogs, metformin, thiazolidinediones, and alpha glycosidase inhibitors. Insulin may be prescribed for type 2 diabetics who fail to achieve glycemic control with other measures. The therapeutic goal for both type 1 and type 2 diabetics is glycemic control, that is, maintaining blood glucose at or near normal concentration levels. Four forms of diabetes have been classified. These four forms are type 1, type II, gestational diabetes, and other specific causes of *DM*. (10)

DM is a chronic disease in which the body does not produce enough insulin to function properly (type 1) or body cells do not react to insulin (insulin resistance) (type 2). Insulin resistance, defined as impaired responsiveness of the body to insulin, is a prediabetic stage associated with obesity, leading to *Type I1 DM*. Oxidative stress is one of major risk factors in diabetes progression. Lifestyle

factors, such as obesity and unhealthy eating habits, as well as increased age disturb redox balance in the body and influence insulin sensitivity. Hyperglycemia exacerbates oxidative stress and leads to the development of microvascular (e.g. retinopathy, nephropathy, and neuropathy) and macrovascular complications (e.g. cardiovascular disease). Complications of diabetes do not only significantly deteriorate diabetic patients' health but also increase the costs of healthcare. [2]

2.1.2 Types of Diabetes Mellitus:

2.1.2.1 Type 1 Diabetes Mellitus:

Type 1 DM is characterized by lack of insulin production and secretion by the beta cells of the pancreas. One cause of the hyperglycemia of *type 1 DM* is an autoimmune destruction of the beta cells of the pancreas. The cell mediated response causes infiltration of the pancreas and reduction in the volume of beta cells. As a protein hormone, insulin acts through chemical responses to receptors on the cells of target tissues. In the muscle, insulin stimulates glucose uptake into cells and enhances glycogenesis. In adipose tissue, insulin stimulates glucose uptake into cells and enhances lipogenesis. In the liver, insulin has a negative effect, inhibiting gluconeogenesis and glycogenolysis. Autoantibodies are present in the circulation of many individuals with *type 1 DM.* There appears to be a genetic susceptibility to development of auto antibodies, with certain histocompatibility antigens predominant in the *type 1 DM* population. However, the development of disease is complex; triggering factors, such as rubella, mumps, and other viral infection, and chemical contact may be necessary for progression of disease. [10]

It was previously called *insulin-dependent DM* or juvenile onset diabetes. β- cells, the only cells in the body that make the hormone insulin that regulates blood glucose. This form of diabetes usually strikes children and young adults, although

disease onset can occur at any age. *Type I DM* accounts for (5-10%) of all diagnosed cases of *DM*. [11]

2.1.2.2 Type II Diabetes Mellitus:

Is characterized by decline in insulin action due to the resistance of tissue cells to the action of insulin. The problem is intensified by the inability of the beta cells of the pancreas to produce enough insulin to counteract the resistance. Thus, type II *DM* is a disorder of both insulin resistance and relative deficiency of insulin. Insulin resistance syndrome, also known as metabolic syndrome and syndrome X, affects the metabolism of many nutrients, including glucose, triglycerides, and high-density lipoprotein (HDL) cholesterol. Individuals who are diagnosed with metabolic syndrome may show abdominal obesity and high blood pressure. Such individuals are at increased risk for cardiovascular disease. The etiology of *type II DM* is complex and multifaceted. There is evidence to show that there is an association of obesity with the development of *type II DM*. Other factors, such as family history of *type II DM* and lack of physical activity, have also been associated with the disorder. Previous diagnosis of gestational diabetes is a risk factor for *type II DM*, as are increasing age, hypertension, and dyslipidemia. Increased risk for developing the disease is also associated with membership in certain racial and ethnic groups, such as African-Americans, Hispanic-Americans, Native Americans, Asian Americans, and Pacific Islanders. [10]

Was previously called *non insulin-dependent DM* or adult onset diabetes. *Type II DM* accounts for about (90-95%) of all diagnosed cases of diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. As the need for insulin rises, the pancreas gradually loses its ability to produce it. Type 2 diabetes is associated with older age, obesity, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity. [11]

2.1.2.3 Gestational Diabetes:

Is similar in etiology to *type II DM*; however, it is defined as diabetes that is diagnosed in pregnancy. Pregnancy is associated with increased tissue cell resistance to insulin. Most pregnant women will compensate with increased secretion of insulin; those individuals who are unable to compensate may develop gestational diabetes (GDM). The hyperglycemia of *GDM* diminishes after delivery; however, the individual who has developed gestational diabetes is at higher risk for the development of type II *DM* thereafter. (10)

2.1.2.4 Other types of Diabetes:

The 4th form of *DM* is termed other specific causes of diabetes. This form of hyperglycemia may be the secondary result of non–insulin-related events. Blood glucose levels are increased in endocrine disorders, such as Cushing's syndrome; in exocrine disorders, such as cystic fibrosis; and as a response to specific drugs, such as protease inhibitors and glucocorticoids. Other causes of this form of diabetes are the result of genetic defects that affect pancreatic beta cells or the action of insulin. The disorders of diabetes differ in their presentation as well as their etiology. Approximately (10%) of diabetics are of the *type 1 DM* variety. The type 1 *DM* disease state usually occurs as acute illness, while *type II DM* progresses slowly over time. (10)

2.1.3 Metabolic features of Diabetes Mellitus:

Patients with *Type 1 DM* tend to be diagnosed before the age of (40 years), are usually lean and have experienced weight loss at the time of presentation. They may with diabetic ketoacidosis. Conversely, patients with type2 diabetes often present later, usually after the age of (40 years), and are often overweight or obese. The presentation can be insidious and they may have had diabetes years before diagnosis. (12)

2.1.3.1 Hyperglycemia:

If plasma glucose concentration exceeds about (10 mmol/L), glycosuria would be expected. High urinary glucose concentrations produce an osmotic diuresis and therefore polyuria. Cerebral cellular dehydration due to hyperosmolality, secondary to hyperglycemia, causes thirst (polydipsia). A prolonged osmotic diuresis may cause excessive urinary electrolyte loss. These 'classic' symptoms are suggestive of diabetes mellitus. *DM* patients on insulin may show the following conditions. The *'dawn' phenomenon* is the physiological response of the elevation of blood glucose concentration in the early morning prior to breakfast due to nocturnal spikes in growth hormone (GH) concentration and a rise in plasma cortisol concentration that increase hepatic gluconeogenesis. Conversely, in some diabetic patients nocturnal hypoglycemia may evoke a rebound counter-regulatory hyperglycaemia called the *Somogyi phenomenon*. Patient blood glucose checking at (02.00– 04.00 hrs), or continuous glucose monitoring if available, may distinguish these conditions, as the *Somogyi phenomenon* reveals hypoglycemia. It is sometimes possible to ameliorate these conditions by giving intermediate-acting insulin before bedtime. [(12)]

2.1.3.2 Abnormalities in lipid metabolism:

These may be secondary to insulin deficiency. Lipolysis is enhanced and plasma non esterified fatty acid (NEFA) concentrations rise. In the liver, *NEFAs* are converted to *acetyl CoA* and ketones, or are re-esterified to form endogenous triglycerides and incorporated into very low density lipoproteins (VLDLs); the latter accumulate in plasma because lipoprotein lipase, which is necessary for *VLDL* catabolism, requires insulin for optimal activity. High-density lipoprotein cholesterol (HDL) concentration tends to be low in *type II DM*. If insulin deficiency is very severe, there may also be chylomicronaemia. The rate of cholesterol synthesis is also increased, with an associated increase in plasma low-

density lipoprotein cholesterol (LDL) concentrations. Consequently, patients with diabetes may show high plasma triglyceride, raised cholesterol and low *HDL* cholesterol concentrations. [12]

2.1.3.3 Long-term effects of Diabetes Mellitus:

Vascular disease is a common complication of *DM*. Macrovascular disease due to abnormalities of large vessels may present as coronary artery, cerebrovascular or peripheral vascular insufficiency. The condition is probably related to alterations in lipid metabolism and associated hypertension. The most common cause of death is cardiovascular disease, including myocardial infarction. Microvascular disease due to abnormalities of small blood vessels particularly affects the retina (diabetic retinopathy) and the kidney (nephropathy); both may be related to inadequate glucose control. *DM* is one of the most common causes of patients requiring renal dialysis. Microvascular disease of the kidney is associated with proteinuria. Kidney disease is associated with several abnormalities, including proteinuria and progressive renal failure. Diffuse nodular glomerulosclerosis (Kimmelstiel–Wilson lesions) may cause the nephrotic syndrome. The presence of small amounts of albumin in the urine (microalbuminuria) is associated with an increased risk of developing progressive renal disease, which may sometimes be prevented by more stringent plasma glucose and blood pressure control. The renal complications may be partly due to the increased glycation of structural proteins in the arterial walls supplying the glomerular basement membrane; similar vascular changes in the retina may account for the high incidence of diabetic retinopathy. Glycation of protein in the lens may cause cataracts. Infections are also more common in diabetic patients, for example urinary tract or chest infections, cellulitis and candida. Diabetic neuropathy can occur, which can be peripheral symmetric sensory, peripheral painful, acute mononeuropathies or autonomic. It has been suggested that sorbitol is implicated in the aetiology of diabetic neuropathy

copper (Cu), chromium (Cr), molybdenum (Mo), selenium (Se) and manganese (Mn), body mass index (BMI), waist to hip ratio (WHR), abdomen circumference (AC) and blood pressure (BP), total cholesterol (TCh), high-density (HDL), low-density lipoprotein (LDL), triglyceride (TG), fasting plasma glucose (FPG), insulin, and Homeostasis Model Assessment—Insulin resistance (HOMA-IR). The men with MS showed statistically significant higher Zn and lower Mg concentrations. Those with diabetes had higher Ca concentration and lower Mg concentration. Cr and Mn concentrations were significantly higher in obese men. The participants with hypertension had lower Mg concentration. We found statistically significant positive correlations (W-TCh, W-LDL, Mg-TCh, Mg-LDL, Ca-TCh, Ca-LDL, Ca-insulin, Ca-HOMAR-IR, Zn-TG, Zn-insulin, Zn-HOMA-IR, Cu-BP systolic, Mn-BMI, Mn-AC, Mn-WHR, Mn-insulin, Mn-HOMA-IR, Se-TCh, Se-LDL, Se-TG, Se-insulin, Se-HOMA-IR, Cr-TCh, Cr-HDL, Cr-LDL, Cr-TG) and negative correlations (Cd-insulin, Hg-WHR, W-insulin, W-HOMA-IR, Mg-BMI, Mg-AC, Mg-WHR, Mg-BP systolic, Mo-insulin, Mn-HDL). Tungsten may contribute to lipid disorders. Magnesium appears to play the protective role in the occurrence of metabolic disorders. Microelements Mn, Cr and Se may intensify MS. [71]

2.3.14 Correlations of Serum Cu+2, Zn+2, Mg+2 and HbA1c in Type 2 and Type 2 Diabetes Mellitus:

Type 2 Diabetes Mellitus (DM) is one of the most frequently seen. Type 2 DM shows identical patophysiological features with Type 1 DM but differs in etiology. There are lots of studies in the area of both types of DM which are influencing body by metabolic and oxidative stress. In this study serum Cu, Zn and Mg levels are determined in three patient groups (Type 1 DM, obese Type 2 DM, non-obese Type 2 DM) and discussed the relationships with serum cholesterol,trigliserid,

glucose and blood HbA1C levels. Study groups evaluated as 21 healty person, 26 non-obese Type 2 DM patients (Body mass index<25), 28 obese Type 2 DM patients (BMI>30), 18 Type 1 DM patients While we didn't find any correlation between parameters of obese Type 2 DM patients, there was slightly negative correlation (r=-0,521, p<0,05) between Cu and Mg, also the same but positive correlation was found between serum Cu and glucose (r=502, p<0,05) levels in non-obese Type 2 DM patients. In Type 1 DM group; there was strong negative correlation (r= -0,604, p<0,05) between serum Cu and Mg levels with a positive strong correlation (r= 0,774, p<0,001) between serum Cu and blood HbA1C levels. In this group there was also strong negative correlation (r=-0,895, p<0,001) with serum Mg ve blood HbA1C . Serum copper levels was increased in all three patients groups, respectively nonobese Type 2 DM, obese Type 2 DM and Type 1 DM group (p<0.05, p=0,001, p<0,001). The meaningful decrease in serum Mg levels (p<0.05) was only found in Type 2 DM group. There were no significant alterations in levels of serum Zn (p>0,05). The results showed that the determination of serum Zn level is not enough to assess the oxidative stress in DM. Because of the probable lacking of serum Zn/Cu antagonism, serum Cu and Mg levels should be determined. In DM, blood HbA1C levels change the profile of trace elements so can be used to assess tha degree of oxidative and metabolic stress. [72]

through the action of aldolase reductase. Erectile dysfunction is also relatively common and in some cases may be partly neurologically mediated. Diabetic ulcers, for example of the feet, can lead to gangrene and amputation. The ulcers can be ischaemic, neuropathic or infective. The joints can also be affected, for example Charcot's joints. Other features of *DM* are skin disorders, such as *necrobiosis lipoidica*, and *abscesses*. [12]

2.1.4 Diagnosis of Diabetes Mellitus:

- Symptoms of hyperglycemia (e.g. polyuria, polydipsia, unexplained weight loss, visual blurring, genital thrush, lethargy) and raised venous glucose detected once - fasting ≥ 126 mg/dL (7 mmol/L) or
- Random ≥ 200 mg/dL (11.1 mmol/L) or
- Raised venous glucose on two separate occasions (fasting ≥ 126mg/dL, random ≥ 200 mg/dL) or
- Oral glucose tolerance test (OGTT) – 2hrs value ≥ 200 mg/dL. [13]

The diagnostic criteria for diabetes mellitus were modified by the Expert Committee to allow for earlier detection of the disease. According to American Diabetes Association (ADA) recommendations, all adults older than age (45 yrs) should have a measurement of fasting blood glucose every (3 yrs) unless the individual is otherwise diagnosed with diabetes. The criteria suggested three methods of diagnosis, each of which must be confirmed on a subsequent day by any one of the three methods.

- Symptoms of diabetes plus a random plasma glucose level of ≥ 200 mg/dL
- A fasting plasma glucose of ≥ 126 mg/dL, or
- An *OGTT* with a 2-hour post-load (75 g glucose load) level ≥ 200 mg/dL. The preferred test for diagnosing diabetes is the measurement of the fasting plasma glucose level. [14]

2.1.5 Glycosylated hemoglobin:

The number of people with diabetes is increasing globally, specially in developing countries, with over (346 million) people diagnosed worldwide. The major hallmark of diabetes is high glucose levels in the blood. However, the concentration of glucose in the blood is not reliable for the diagnosis of diabetes because of fluctuations in these concentrations throughout the day. The relative concentration ratio of *Hemoglobin A1c (HbA1c)* to *Hemoglobin (Hb)* is a reliable biomarker for the diagnosis and prognosis of diabetes. *HbA1c* is produced by a non-enzymatic reaction between glucose and the *N*-terminal valine of the β-chain of hemoglobin in red blood cells. Since the half-life of red blood cells is approximately (2 months), the concentration of *HbA1c* represents the blood glucose levels over the past (2–3 months). Since the first quantitative assay of HbA1c, the assay method has been improved by a rapid automated assay and an automated immunoassay. Conventional *HbA1c* assay methods available to clinical laboratories are based on the chromatographic method. The chromatographic method, involving the use of *High performance liquid chromatography (HPLC)* for the separation of *HbA1c* and *Hb*, is rather expensive. [15]

2.1.6 Candidate genes influencing Diabetic susceptibility:

It is clear that there is a genetic element to the development of *Type 1 DM* although the association is much less strong than in *Type I1 DM*. *DM* in *United Kingdom (UK)* estimates that if a mother has the condition, the risk of the child developing T1DM is about (2%) whereas if the father is diabetic then the risk to the child is estimated to be (8%). If both parents suffer from *Type 1 DM* then the offspring will have a (30%) chance of developing the condition. [16]

For *Type 1 DM*, the concordance rate for monozygotic twins from a number of studies has been estimated as (21-53%), with most estimates being between (30-50%). Candidate gene studies and genome wide analysis have revealed that the

most important loci are in the *Human leukocyte Antigen (HLA)* class II region on chromosome 6p21. This region accounts for (50%) of familial aggregation through protective and detrimental effects. [16]

2.2 Trace elements:

Many trace elements, among which metals, involved as cofactors in myriads of biochemical-specially-enzymatic reactions. As such they play cardinal roles in many physiological processes, in particular immunity and metabolism. A good example to illustrate their important contribution is magnesium: low magnesium levels have been associated with increased *type II DM,* whereas controversy exists about the importance of hypomagnesemia in prediabetic states. [3]

Trace elements have been identified for long time as potential candidates for improving metabolic disorders like prediabetes (insulin resistance, obesity, metabolic syndrome) or *DM*. In parallel with increasing comprehension of cellular and biochemical mechanisms leading to-or aggravating- these metabolic disorders, identifying the cellular targets and sites of action of trace elements has reactivated interest in their therapeutic potential. Activation of insulin receptor signalling *(chromium Cr),* antioxidant properties *(Se), (Zn)* or inhibition of phosphatases *(vanadium Va)* thus appeared promising in view of the key importance of these processes in glucose homestasis and insulin sensitivity. Indeed insulin receptor/postreceptor signalling defects are considered to underlie glycemic dysregulation and insulin resistance, although the precise causal defects must still be unraveled. Prediabetic states, and even more so frank *DM*, are characterized by inflammation (cytokines) and oxidative stress, due to disruption of the equilibrium between production of free radicals and their scavenging by multiple antioxidant systems. Moreover, these mechanisms may be involved in concert in the pathogenesis of insulin resistance and accompanying pathologies. [3]

Trace elements present in very small amounts in the living tissues but are important for the vital processes of life. [17] Many trace elements are indispensable for proper functioning of a myriad of biochemical reactions, more particularly as enzyme cofactors. This is particularly true for the vast set of processes involved in regulation of glucose hemostasis, being it in glucose metabolism itself or in hormonal control, specially insulin. [3] Some metals are known as macro-metals and are found in high amount in the body tissues, therefore they are also called macro-nutrients. [18] At least (100 mg) of each macro-nutrient is required in the daily diet. [19] In contrast, some metals e.g. *(Cu), (Zn), (Cr), iron (Fe) and manganese (Mn) etc*. are needed in the body in very small amounts, less than (100 parts) per million, hence, these are called trace elements or micro-nutrients. [20] Metals are involved in a range of physiological processes such as prosthetic groups of many proteins, water balance, cofactors of many enzymes etc. [21] Several metals function as part of proteins/enzymes as metalloproteinase/metalloenzymes. [22] Such proteins without metal containing prosthetic groups are unable to perform their physiological functions. [23] The regulation of various metallic contents in the body is pre-requisite for their proper functioning. [24] Metals enable the muscles to contract or relax, and also transmit impulses through the nerves. Most metals are available in the soluble salt forms, which regulate the composition of biofluids. The proper metabolic functioning of the trace elements depends on their normal levels in various body tissues. [25] Due to the diversified metabolic characteristics and functions; various metals such as *(Mg), (Zn), (Cr), (Fe), (Mn) and (Cu)* are considered as essential for normal human health. [17]

Several studies have reported that the imbalance of some essential metals might adversely affect pancreatic islet and cause development of diabetes. [26] It is also manifested that some *Reactive Oxygen Species (ROS)* are produced during diabetes due to imbalance of essential metals. This oxidative stress might decrease the

insulin gene promoter activity and mRNA expression in pancreatic islet cells due to hyperglycemic condition. [27]

On contrary to essential metals, some toxic metals have also been identified which accumulate in various biological samples of *Type II DM* patients. Uncontrolled pollution and industrialization might be a potential source to expose human population against toxic metals such as *lead (Pb), nickel (Ni), cadmium (Cd) and arsenic (As).* Some of the toxic metals are implicated to disrupt the glucose uptake and alter the related molecular mechanism in glucose regulation. [28]

(Zn), (Se), (Cu) and molybdenum are involved in many biochemical processes supporting life. The most important of these processes are cellular respiration, cellular utilization of oxygen, *DNA* and *RNA* reproduction, maintenance of cell membrane integrity, and sequestration of free radicals. *(Zn), (Se)* and *(Cu)* are involved in destruction of free radicals through cascading enzyme systems. Superoxide radicals are reduced to hydrogen peroxide by superoxide dismutases in the presence of *(Zn)* and *(Cu)* cofactors. Hydrogen peroxide is then reduced to water by the selenium-glutathione peroxidase couple. Efficient removal of these superoxide free radicals maintains the integrity of membranes, reduces the risk of cancer, and slows the aging process. On the other hand, excess intake of these trace elements leads to disease and toxicity; therefore, a fine balance is essential for health. Trace element--deficient patients usually present with common symptoms such as malaise, loss of appetite, anemia, infection, skin lesions, and low-grade neuropathy, thus complicating the diagnosis. Symptoms for intoxication by trace elements are general, for example, flu-like and *Central Nervous System (CNS) symptoms, fever, coughing, nausea, vomiting, diarrhea, anemia, and neuropathy*. A combination of observation, medical and dietary history, and analyses for multiple trace elements are needed to pinpoint the trace element(s) involved. [29]

2.2.1 Classification of trace elements:

2.2.1.1 WHO Classification:

- Essential elements.
- Probably essential elements.
- Potentially toxic elements.

2.2.1.2 Frieden's Classification

- In 1981, Frieden proposed a biological classification of trace elements based on their amount in tissues:
- Essential trace elements: boron, cobalt, copper, iodine, iron, manganese, molybdenum, and zinc.
- Probably essential trace elements: chromium, fluorine, nickel, selenium, and vanadium.
- Physically promotive trace elements: bromine, lithium, silicon, tin, and titanium.

2.2.2 Physiology of trace elements:

2.2.2.1 Copper:

Copper is the third most abundant trace element with only (75–100 mg) of total amount in the human body. *(Cu)* is present in almost every tissue of the body and is stored chiefly in the liver along with the brain, heart, kidney, and muscles. *(Cu)* is absorbed in the gut and transported to the liver. In human blood, *(Cu)* is principally distributed between the erythrocytes and in the plasma. It is transported in the form of ceruloplasmin into the plasma where its metabolism is controlled and is excreted in bile. Ceruloplasmin accounts for (90%) of the *(Cu)* content in blood and is responsible for carrying copper to the deficient cells. Copper-zinc metalloenzyme superoxide dismutase contains (60%) of the copper in erythrocytes and the remaining (40%) is loosely bound to other proteins and amino acids.[7]

(Cu) is essential mineral, which is needed for several biological functions. It is required for the catalytic activity of *superoxide dismutase (SOD)* that participates in the protection of cells from superoxide radicals. [30] *(Cu)* imbalance is implicated in cholesterol elevation by disrupting normal *(HDL)* and *(LDL)* balance. [31] *(Cu)* also activates cytochrome oxidase which is involved in the electron transport chain of the mitochondria. [32] In case of copper deficiency, cytochrome oxidase reduces its activity which might lead to the distortion of mitochondria in metabolically active tissues such as pancreatic acinar cells, hepatocytes etc. [33]

(Cu) deficiency is one of the reasons for the development of cardiovascular diseases. [34] Other reports suggest that *(Cu)* is also beneficial to prevent arthritis associated inflammation and epilepsy. [35] More recently, it has been reported that disturbances in copper levels in various biofluids and tissues are associated with abnormalities implicated in metabolic pathways of diabetes and its complications.[17] *(Cu)* as well as zinc metals play roles in order to protect oxidative damage of body tissues. [36]

(Cu) has an integral role in many enzymatic activities involved in modifying oxidative stress. Free *(Cu)* ions have catalytic activity in the generation of highly reactive hydroxyl radicals. Disruption of *(Cu)* hemostasis induces oxidative damage by free radicals; such *(Cu)* toxicity is associated with disrupted lipid metabolism, hepatic disorders, neurodegenerative disorders, and atherogenesis. *(Cu)* ion may also play a protective role in the accumulation of human islet amyloid peptide, which is the major component of amyloid deposits in pancreatic β-cells of *type II DM* patients; however, whether or not *(Cu)* have a protective role in the etiology of *type II DM* is not clarified. Excess of *(Cu)* under inflammatory conditions trigger oxidative stress which are present in chronic diseases. On the other hand, increased (*Zn)* ion levels may provide a protective effect against

(Cu)toxicity by competing for *(Cu)* binding site. [5] The biological functions of copper have been listed:

- The enzyme cytochrome c oxidase, comprising *(Cu)* and iron, plays a vital role in energy production during aerobic respiration.
- *(Cu)* is also present in superoxide dismutase which detoxifies superoxide's by converting them to oxygen and hydrogen peroxide.
- *(Cu)* is also a component of lysyl oxidase which takes part in the synthesis of collagen and elastin. *(Cu)* is also essential for maintaining the strength of the skin, hair, blood vessels, and epithelial and connective tissue throughout the body.
- *(Cu)* plays a considerable role in the production of hemoglobin. Ceruloplasmin catalyzes the oxidation of iron which subsequently is necessary to bind to its transport protein, transferrin.
- Melanin production: *(Cu)* containing enzyme tyrosinase converts tyrosine to melanin.
- Myelin production: *(Cu)* is also necessary for the synthesis of phospholipids found in myelin sheaths in peripheral nerves.
- *(Cu)* is also required for the production of the thyroid hormone thyroxine.
- *(Cu)* can act as both an antioxidant and a prooxidant. As an antioxidant, *(Cu)* scavenges or neutralizes free radicals and may reduce or help prevent some of the damage they cause. *(Cu)* promotes free radical damage to the tissues when it acts as prooxidant. [7]

The symptoms of *(Cu)* deficiency are *hypochromic anemia, neutropenia, hypopigmentation of hair and skin, abnormal bone formation with skeletal fragility and osteoporosis, joint pain, lowered immunity, vascular aberrations, and kinky.* Deficiency of *(Cu)* in diet for a prolonged period especially during stages of active

growth leads to anemia and defective keratinisation in the oral cavity. The anemic effect is attributed to decreased ferroxidase activity of ceruloplasmin and reduced iron oxidation. Lowered immunity can result in various infections of the oral cavity due to accompanied neutropenia. Granulocyte maturation disorder in the bone marrow and vacuolation in neutrophils have been noted. Bone changes in copper deficiency include a loss of trabecular formation with thinning of the cortex. There may be osteoporosis and occipital horn formation due to functional impairment of copper-requiring enzymes in case of copper deficiency. Various studies found that the mean serum copper levels were significantly higher in the sera of patients with oral potentially malignant disorders. *(Cu)* is reportedly present in the saliva for as long as (30 mins). The longer the presence of *(Cu)* in saliva, the higher the chances of its uptake by the oral epithelium. It has been advocated that copper appears in the blood after (15 mins) of ingestion of areca nut and its products. In oral submucous fibrosis patients, the serum levels of *(Cu)* gradually increase as the clinical stage of the disease progresses. However, local effect of raised salivary copper may have a more important role to play than the raised serum levels. Other schools of thoughts appraised decrease in the *(Cu)* serum concentrations due to usage of copper in upregulation of lysyl oxidase leading to excessive cross linkage of collagen. *(Cu)* is also believed to possess caries promoting property. [7]

2.2.2.2 Zinc:

Zinc *(Zn)* is an essential trace element which is required for normal cell processing e.g. cell division and apoptosis. *(Zn)* participates in multiple biochemical pathways such as in transcription, translation and cell divisions. [37] More than (300) enzymes need *(Zn)* for their catalytic activities. On the other hand, removal of *(Zn)* from catalytic site leads to the loss of enzymatic activity. [38]

About (70%) of the *(Zn)* is bound to albumin and any pathological alteration of albumin affects the serum Zn levels [39]. *(Zn)* malabsorption results in various

types of disorders including the dermal, gastrointestinal, neurological and immunological abnormalities. [40]

There is (2–4 g) of *(Zn)* distributed throughout the human body. *(Zn)* is stored in prostate, parts of the eye, brain, muscle, bones, kidney, and liver. It is the second most abundant transition metal in organisms after iron and it is the only metal which appears in all enzyme classes. In blood plasma, *(Zn)* is bound to and transported by albumin (60%) and transferrin (10%). Since transferrin also transports iron, excessive iron can reduce *(Zn)* absorption, and vice versa. The concentration of *(Zn)* in blood plasma stays relatively constant regardless of *(Zn)* intake. [7]

Recently, published studies revealed that *type II DM* patients have suboptimal *(Zn)* status in blood due to its increased urinary depletion. As a result, hypozincemia and *hyperzincuria* are developed in diabetics. *(Zn)* plays a key role in the storage and secretion of insulin, which subsequently increases the uptake of glucose. [17] The decreased plasma level of *(Zn)* adversely affects the ability of islet cells to produce and secrete insulin. [41]

It is well established that *(Zn)* transporter *(ZnT8)* is a key protein for the regulation of insulin secretion from the pancreatic β-cells. Recently a mutation in (*ZnT8)* transporter has been associated with *T II DM*. [42] Briefly all these evidences show the importance of *(Zn)* in the maintenance and integration of insulin hexamer and its role in the metabolic regulation. [41]

(Zn) is an essential micronutrient which has an important role in the functioning of hundreds of enzymes, in insulin metabolism and acts as an efficient antioxidant. Consequently many observations related to *(Zn)* deficiency, although clinical signs of deficiency are quite modest. *(Zn)* is found mainly in cereals, meat, seafood and dairy products. Although usual intakes of *(Zn)* are harmless; the range between safe and unsafe is relatively narrow. Because *(Zn)* has no storage form, there is

need for a constant supply and *(Zn) availability* to cells is particularly well regulated, albeit poorly understood. *(Zn) absorption* can be reduced by iron or inflammatory bowel diseases. It is transported across cell membranes via two families of transporters. Over (20) transporters are identified and most are intracellular. Concerning metabolic diseases *(insulin resistance, metabolic syndrome, diabetes), (Zn)* is considered important mainly because it plays a major role in the stabilization of insulin hexamers and the pancreatic storage of the hormone and it is an efficient antioxidant, while oxidative stress is considered to be a main component in initiation and progression of insulin resistance and diabetes.

Severe *(Zn)* deficiency is not frequent but concerns have nevertheless been raised about *(Zn)* levels in diabetic patients because of increased excretion due to polyuria. Whereas some studies have reported *(Zn) deficiency* in *type II DM,* others failed to find significant differences with healthy subjects. *In type 1 DM*, *(Zn) deficiency* is expected to increase the pancreatic damage .A recent report described reduced levels of *(Zn)* in obese, insulin resistant subjects. Lower *(Zn)* plasma concentrations were found in *type II DM* but they were not related to glycemic status or *DM* duration or components of the *metabolic syndrome*. Interestingly it was reported that diabetic have elevated levels of *(Cu)* and it could be that copper is in fact linked to metabolic syndrome and diabetes. Nevertheless, reduced *(Zn)* levels in diabetics appear to be related to increased risk for coronary artery disease and mortality.[(3)]

Several modes of action have been described to explain the improved action of insulin by *(Zn)*. It appears that *(Zn)* can have direct insulin-like effects, which may be due to inhibition of the important glycogen-regulating enzyme. Other mechanisms include stimulation of the postreceptor proteins. *(Zn)* can also reduce cytokines and induces metallothionein synthesis, whereby *(Zn)* may have indirect efficacy. Finally it has been suggested that the important *(Zn)* concentrations for its

action are the intracellular -rather than plasma- levels. *(Zn)* is a remarkable antioxidant: it acts at specific sites where it can compete for iron and copper; it further binds to SH groups in proteins, protecting them from oxidation. Because *(Zn)* controls metallothionein expression it is involved in cellular redox regulation. Oxidative stress can be corrected by dietary *(Zn)* as demonstrated by an elevation of hepatic antioxidant enzymes. [3]

High *(Zn)* slightly reduced the risk for *type II DM* in a prospective study performed in (8300) women and the effect was limited to the *Zn-deficient* subgroup .Another study, however, reported aggravation of glucose intolerance in *Zn-deficient* diabetic patients. In microalbuminuric *type II DM* patients *(Zn)* lowered homocysteine levels while increasing *vitamin* B_{12} and folate levels. In obese, non-diabetic subjects insulin sensitivity was improved without changes in leptin levels. However no preventive effect against diabetes development could be found in obese women. Oxidative stress was reduced by *(Zn)* in healthy elderly subjects.

It increases the pancreatic insulin content and improves the glucose tolerance test. Its levels are lower in obese human subcutaneous and visceral adipose tissue and liver, but interestingly does not appear to be related to insulin resistance. [3]

(Zn) is a critical trace element in human health. *(Zn)* n has a potential to be utilized for the treatment of *type II DM* however, the epidemiologic evidence suggests that the effect of *(Zn)* on *type II DM* remains unclear. Up to (85%) of the whole body *(Zn)* content is found in muscle and bones, with (11%) in the skin and liver. *(Zn)* is an indispensable cofactor for more than (300) enzymes involved in metabolism and also reportedly plays a role in aging, immune system, apoptosis, and oxidative stress. Although the effect of *(Zn)* supplementation in the improvement of oxidative stress is controversial, one of the causes that the oxidative stress is present in patients with *type II DM* is the change in *(Zn)* metabolism. Recent studies have demonstrated that the islet-restricted *(Zn)* transporter, regulates insulin

secretion and hepatic insulin clearance, suggesting that *(Zn)* is a key biological factor in glucose hemostasis and the risk of developing *type II DM*. [5]

(Zn) functions in biology are numerous but can be separated into three main categories: catalytic, regulatory, and structural roles. It is required for the catalytic activity of a large number of enzymes. It plays an important role in immune function, wound healing, protein synthesis, *DNA* synthesis, and cell division. *(Zn)* is required for proper sense of taste and smell. It also supports normal growth and development during pregnancy, childhood, and adolescence. Allegedly, it also possesses antioxidant properties and thus may play a role in speeding up the healing process after an injury and protecting against accelerated aging. *(Zn) ions* are effective antimicrobial agents even at low concentrations. [7]

2.2.2.3 Iron:

Iron is the most abundant essential trace element in the human body. The total content of iron in the body is about (3–5 g) with most of it in the blood and the rest in the liver, bone marrow, and muscles in the form of heme. *(Fe)* is absorbed in the gut from diet in case of depletion and transported in the form of ferritin. Hemosiderin is a golden brown pigment which is a byproduct of metabolism of ferritin and is deposited in the cells of the reticuloendothelial system. [7]

Heme is the major iron containing substance in ferrous or ferric state which is present in hemoglobin, myoglobin, and cytochrome. Heme forms covalent bonds with the globin protein to form hemoglobin which is the major oxygen carrying pigment in *RBCs* of mammalians. It takes part in a myriad of metabolic cycles such as in the energy producing reactions (the cytochromes of the Krebs cycle) in all the cells and activates the energy producing oxidizing enzymes. Apart from participation in maintaining innumerable physiological and metabolic processes, it is also necessary for *DNA*, *RNA*, collagen, antibody synthesis, and so forth. The

biological roles of iron in the human body are beyond the scope of this paper and only few important ones have been listed. [7]

(Fe) is an essential transition metal required for the synthesis of two important functional proteins such as hemoglobin and myoglobin, which are involved in the transport of molecular oxygen during respiration. [43] In blood stream small fraction of serum *(Fe)* is transported by a glycoprotein, called transferrin into the cells. [44] In the body tissues, ferritin stores free *(Fe)*, which is increased in newly diagnosed diabetic subjects. [45] Recently, a report showed a positive correlation between serum ferritin and Fe deposition in tissues, which linearly increased with diabetes duration. [46] The serum ferritin elevation is regarded as an index of *(Fe) overload*, which successively leads to a condition called hemochromatosis. [47] Several studies showed association between hemochromatosis and *Type II DM*. [48] The elevated *(Fe)* level oxidizes various biomolecules such as nucleic acids, proteins and lipids, which may contribute to *Type II DM* development by decreasing insulin secretion from pancreatic beta cells with concomitant increase of insulin resistance.[49] serum ferritin level might become a surrogate marker of diabetes to predict disease onset. [50]

Iron deficiency anemia is the most common manifestation of low serum levels of this important trace element. *Microcytic hypochromic RBCs*, fatigue and lowered memory are some of the features of *iron deficiency anemia*. It has also been noted that serum ferritin levels are elevated and serum iron concentrations are decreased with tumor progression in head and neck carcinomas and thus heme can be used as a follow-up tool for patients along with nutritional assessment. [7]

2.2.2.4 **Magnesium:**

Magnesium is essential for the maintenance of proper health. It is required for the activity of more than (300) enzymes, which serve several important physiological

functions in the human body. [51] *(Mg)* containing enzymes are involved in the glucose hemostasis, nerve transmission, *DNA* and *RNA* production. [52]

(Mg) deficiency might lead to a decrease in insulin mediated glucose uptake. [53] On the other hand, *(Mg)* supplementation prevented insulin resistance and also reduced the development of diabetes. Some studies reported low level of *(Mg)* in the blood serum and an increased urinary excretion of *(Mg)* in the diabetics relative to their healthy control subjects. [53]

2.2.2.5 Manganese:

Manganese acts as a cofactor in several enzymes including those involved in bone marrow production, and metabolism of carbohydrates, proteins and fats. [54] It is essential for the proper utilization of *vitamin C and vitamin E. (Mn)* as a cofactor of enzymes is also involved in mitochondrial glycoproteins synthesis. [55]

Impaired activity of these enzymes, due to *(Mn)* deficiency leads to abnormal cartilage production. [56] *(Mn)* is also a cofactor of pyruvate carboxylase, which plays a role in the conversion of various non-carbohydrate compounds into glucose via gluconeogenesis for their subsequent use. In short, *(Mn)* is also required for normal insulin synthesis, its secretion, and an alteration in its metabolism has been implicated in diabetes development. [17] Very recently, in an elegant study reported Mn deficiency in *type II DM* with respect to their control subjects. [57] Low levels of manganese are associated with epilepsy. *(Mn) deficiency* was suggested as an underlying factor in hip *abnormalities, joint disease*, and *congenital malformation*. Manganese deficiency can cause heart and bone problems. [58]

2.2.2.6 Selenium:

Selenium is relatively well absorbed from diet, better so if it is in an organic form. It acts as an antioxidant in the form of selenoproteins; Selenoproteins are also responsible for the transport of *(Se)* to tissues. Severe *(Se)* deficiency is rare, while reduced *(Se)* levels are seen in diabetics together with increased oxidative stress. In

offsprings of diabetic patients *(Se)* correlates inversely with (*CRP)* and with insulin resistance. In view of the potent antioxidant and anti-inflammatory effects of *(Se)* and the prominent role played by these disorders in insulin resistance and *DM*. [3]

2.2.2.7 Chromium:

The total body content of *(Cr)* is relatively low in an average healthy human adult. *(Cr)* is an essential trace element and plays an important role in glucose metabolism by serving as a cofactor for insulin action. *(Cr)* is excreted principally in the urine and faeces and in small quantities in the hair, sweat, and bile. [7]

(Cr) has high biological activity which is required for the optimal glucose uptake by cells. (59) *(Cr)* regulates insulin and blood glucose levels by stimulating insulin signaling pathway and metabolism. [59]

(Cr) deficiency results in the elevation of blood glucose levels and if it is persisted for a prolonged period, it may lead to the development of diabetes. [3] Some reports show that Cr supplements decrease the blood sugar level in diabetes. [60] Prolonged hyperglycemia increases *(Cr)* urinary excretion. Their imbalance predisposes to glucose intolerance which subsequently converts to *DM* related complications. [17]

The importance of chromium *(Cr)* for glucose metabolic regulation has been seen in clinical states of relatively severe *(Cr) deficiency*, characterized by impaired glucose tolerance, fasting hyperglycemia and eventually lipid disorders. *(Cr)* can bind directly to insulin, in particular to insulin dimers, thereby possibly stabilizing the hormone structure and/or modifying its receptor binding. [3]

2.2.2.8 Vanadium:

Vanadium *(Va)* has been known for about one century to possess *anti-diabetic* properties, is poorly absorbed in its inorganic form. However only over the past two decades have concrete therapeutic applications been performed. [3]

2.2.2.9 Lithium:

Lithium *(Li)* is a complex trace element mainly used in neurological disorders and more particularly for treating mood and bipolar disorders. Its clinical management is difficult and *(Li)* is likely toxic. Transient diabetes has been associated with *(Li)* withdrawal, pointing to the potential effect of this trace element. [3]

2.2.2.10 Cobalt:

Cobalt is an essential trace element for the human body and can occur in organic and inorganic forms. it forms an integral part of *vitamin* B_{12}. [7]

2.2.2.11 Molybdenum:

Molybdenum, as a component of molybdoprotein, takes part in the formation of active sites for various enzymes. A molybdenum-containing enzyme has some role to play in purine catabolism. It also influences protein synthesis and growth of the body. Molybdenum has an antagonistic effect against copper; thus, high concentrations of molybdenum can reduce copper absorption and subsequently. [7]

2.2.2.12 Fluorine:

Fluorine makes negligible part of body weight and enters the system principally through drinking water and to a lesser extent through foods. Fluorine, in the form of fluorapatite crystals, is an important part of the organized matrix of hard tissues like bone and teeth. It is also believed that fluoride, in combination with calcium, stimulates osteoblastic activity. [7]

2.2.2.13 Iodine:

Iodine is a vital trace element required at all stages of life especially during formative years. It is important to sustain the daily functions of human body and deficiency or excess can have significant adverse effects on the body. Iodine is an essential component of thyroid hormones, that is, tetraiodothyronine (*T4* or thyroxine) and triiodothyronine *(T3).* It plays a significant role in the functioning of the parathyroid glands. Iodine plays an important role in general growth and

development of the body along with maintaining metabolic processes. There can be innumerable symptoms of iodine deficiency or excess. The deficiency of iodine is more commonly evident. Most commonly reported symptoms of iodine deficiency are *extreme fatigue, irritability, mental disturbances, weight gain, facial puffiness, constipation, and lethargy*. [7]

2.2.3 Toxic metals:

Toxic metals e.g. *(Pb), (Ni), (Cd)* and *(As)* deposit in tissues and are non-degradable. Hence, these metals remain in the tissues for a long period, and it is often difficult to eliminate metal-based problem. Body tissues can tolerate a certain level of metals, and beyond such threshold limits tissues get damaged due to metal toxicity. Furthermore, essential metals also have carcinogenic effects if present in excess amounts than they are required. Preponderance of these toxic metals in the environment is potentially alarming and harmful for human health are common in the nature and present in air, water and soil, which increases the probability of human exposure. [61]

Toxic metals react with various proteins in the body that may modify their functions and kinetics. Moreover, when diet is low in essential metals, the body absorbs and makes use of more toxic metals. In the current environmental conditions several human populations are exposed to high levels of toxic metals including *(Pb), (Cd), (As) and (Ni)* .An abundance of a toxic metal competes with essential metal for enzymes activity and various body physiological functions .For example, *(Zn)* is required for the activity of many enzymes. In case of *(Zn)* deficiency and increased exposure to toxic metals such as lead *(Pb)*, body will use Pb instead of *(Zn).* Some toxic metals including *(Pb), (Cd), (As) and (Ni)* are elevated in biological samples of diabetic patients, which adversely affect health status of an individual by disrupting organ physiology and functions. (*Free Pb)* in blood plasma is rapidly transferred to soft body tissues. [61]

2.2.4 Trace element supplementation:

Trace element supplementation as monotherapy is of weak efficacy in diabetes and hardly efficacious in prediabetic states. The global outcome in humans is somewhat frustrating in view of the logical good expectations from mechanistic effects of these metals at key steps of glucose and insulin regulatory control. Another concern is the relatively narrow range between safe and unsafe doses for oral treatment of human individuals. [3]

Finally trace elements may also be of particular interest in pre/postnatal periods since deficiencies during pregnancy have negative consequences on several constitutive and metabolic parameters. [3]

2.2.5 Detection of Trace Elements:

Though various methods have been employed to determine the presence of trace elements, it is a cumbersome due to their wide distribution within the living tissues and enzyme systems. Colorimetric and spectrographic methods are used commonly to analyse the amount of trace elements. Typically, spectroscopy and electrochemical methods are preferred for solitary element analysis whereas neutron activation analysis and spectroscopic methods are used for determination of more than one element. [7]

Serum, plasma, and erythrocytes may be used for the evaluation of copper and zinc status, whereas only serum or plasma is recommended for selenium. Whole blood is preferred for molybdenum. When trace element levels are inconsistent with medical evaluations, a test for activity of the suspected enzyme(s) would support the differential diagnosis. Furthermore, it is important to differentiate whether trace element deficiency or toxicity is the primary cause of the disorder, or is secondary to other underlying diseases. Only successful treatment of the primary disorder will lead to complete recovery. Royal blue top evacuated tubes containing negligibly low concentrations of the trace element or acid-washed plastic sterilized syringes

should be used for blood, serum, or plasma collection. Powdered gloves must be avoided. When possible, mineral supplements are not to be administered to the patient for a minimum of (3 days) prior to sample collection. Serum and plasma specimens are to be transported in acid-washed polypropylene and polyethylene tubes. Analysis is performed in a controlled environment to minimize or eliminate contamination. During analysis, all laboratory wares should be acid-washed for decontamination. *(Cu)* and *(Zn)* analysis on serum and plasma are commonly performed by flame atomic absorption spectrometry, inductively coupled plasma-atomic emission spectrometry, and inductively coupled plasma-mass spectrometry. Serum and plasma selenium levels are determined by graphite furnace atomic absorption. Molybdenum levels are best determined by neutron activation and highly sensitive inductively coupled plasma-mass spectrometry. [29]

The most easily determined deficiency is of iron which can be determined by performing laboratory tests. Recently, newer approaches like erythrocyte zinc porphyrin assay have also been used in primary screening tests for assessing iron status. [7]

The reported optimal plasma or serum ratio between *(Cu)* and *(Zn)* is (0.7–1.0). Severe (*Cu deficiency)* can be found by testing for low plasma or serum *(Cu)* levels, low ceruloplasmin, and low superoxide dismutase levels but these are not very sensitive tests and fail to determine marginal *(Cu* deficiency). [7]

The assessment of the iodine nutritional status of a population or group living in an area or region suspected to be iodine-deficient area can be performed by assessment of the goitre rate, measurement of urinary iodine excretion, and determination of the level of blood *(T3), (T4), or (TSH).* [7]

Tissue chromium stores apparently do not truly reflect the blood chromium; thus, serum chromium concentration is not a good indicator of chromium status.

Excessive exposure of individual to chromium via occupation or accident may be reflected by elevated serum chromium. [7]

Different tissues such as blood, hair, and nails have been analyzed for determining the nutritional status of selenium. Generally, these tissues can provide a sound appraisal of selenium status if dietary *(Se)* intake is relatively uniform. Tissue levels status of other trace elements in normal individuals is difficult to determine.[7]

2.3 Previous studies:

2.3.1 Concentrations of seven trace elements in different hematological matrices in patients with type 2 diabetes as compared to healthy controls:

This study aimed to compare the trace element status of patients with type 2 diabetes (n=53) with those of nondiabetic healthy controls (n=50). The concentrations of seven trace elements were determined in the whole blood, blood plasma, erythrocytes, and lymphocytes of the study subjects. Vanadium and iron levels in lymphocytes were significantly higher in diabetic patients as compared to controls (p<0.05 for iron and p<0.01 for vanadium). In contrast, lower manganese (p<0.01) and selenium (p<0.01) concentrations were detected in lymphocytes derived from patients with type 2 diabetes versus healthy subjects. Furthermore, significantly lower chromium levels (p<0.05) were found in the plasma of diabetic individuals as compared to controls. Trace element concentrations were not dependent on the degree of glucose control as determined by correlation analysis between HBA_{1c} versus metal levels in the four blood fractions. In summary, this study primarily demonstrated that trace element levels in lymphocytes of patients with type 2 diabetes could deviate significantly from controls, whereas, in general, no considerable differences could be found when comparing the other fractions between both patient groups. Therefore, it seems reasonable to analyze metal levels

in leukocytes to determine trace element status in patients with type 2 diabetes and perhaps in other diseases. [62]

2.3.2 Copper, Chromium, Manganese, Iron, Nickel, and Zinc Levels in Biological Samples of Diabetes Mellitus Patients:

There is accumulating evidence that the metabolism of several trace elements is altered in diabetes mellitus and that these nutrients might have specific roles in the pathogenesis and progress of this disease. The aim of present study was to compare the level of essential trace elements, chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn) in biological samples (whole blood, urine, and scalp hair) of patients who have diabetes mellitus type 2 ($n = 257$), with those of nondiabetic control subjects ($n = 166$), age ranged (45–75) of both genders. The element concentrations were measured by means of an atomic absorption spectrophotometer after microwave-induced acid digestion. The validity and accuracy was checked by conventional wet-acid-digestion method and using certified reference materials. The overall recoveries of all elements were found in the range of (97.60–99.49%) of certified values. The results of this study showed that the mean values of Zn, Mn, and Cr were significantly reduced in blood and scalp-hair samples of diabetic patients as compared to control subjects of both genders ($p < 0.001$). The urinary levels of these elements were found to be higher in the diabetic patients than in the age-matched healthy controls. In contrast, high mean values of Cu and Fe were detected in scalp hair and blood from patients versus the nondiabetic subjects, but the differences found in blood samples was not significant ($p < 0.05$). These results are consistent with those obtained in other studies, confirming that deficiency and efficiency of some essential trace metals may play a role in the development of diabetes mellitus. [17]

2.3.3 Copper, zinc and magnesium levels in type-1 diabetes mellitus:

Alterations in plasma concentrations of several trace elements have been reported to occur in type-1 diabetes mellitus. These micronutrients are suspected to have a role in pathogenesis and progression of the disease.

In a comparative analysis, the plasma concentration of copper, zinc and magnesium was estimated in 37 patients with type-1 diabetes mellitus and 25 healthy non-diabetic subjects at Sher-i-Kashmir Institute of Medical Sciences, Srinagar, Kashmir, India. Trace elements were estimated using a GBC 902 double beam atomic absorption spectrophotometer.

Mean plasma concentrations of copper and magnesium were comparable between diabetic patients and control subjects. Plasma zinc levels were significantly higher (P=0.022) in diabetic patients (17.78 0.6 micromol/L) as compared to controls (15.80 0.75 micromol/L). Glycemic control and presence of microalbuminuria did not influence the plasma levels of copper, zinc and magnesium.

Plasma zinc levels are significantly higher in type-1 diabetes mellitus patients, while plasma copper and magnesium levels are not significantly altered. No effect of sex, glycemic control or presence of microalbuminuria could be demonstrated on plasma concentration of trace elements in type-1 diabetes mellitus patients. [63]

2.3.4 Copper, zinc, and magnesium levels in non-insulin dependent diabetes mellitus:

A relationship has been reported between trace elements and diabetes mellitus. This study evaluated the role of such a relationship in 83 patients with non-insulin dependent diabetes mellitus (40 men and 43 women), with a mean duration of diabetes of 3.9 +/- 3.6 years. Patients with nephropathy were excluded. Thirty healthy non-diabetic subjects were studied for comparative analysis. Subjects were subdivided into obese and non-obese. Diabetic subjects were also subdivided into controlled and uncontrolled groups; control was based on fasting blood glucose and serum fructosamine levels. Plasma copper, zinc and magnesium levels were

analysed using a GBC 902 double beam atomic absorption spectrophotometer. Plasma zinc and magnesium levels were comparable between diabetic and non-diabetic subjects, while copper levels were significantly elevated ($p < 0.01$) in diabetic patients. Age, sex, duration and control of diabetes did not influence copper, zinc, or magnesium concentrations. We conclude that zinc and magnesium levels are not altered in diabetes mellitus, but the increased copper levels found in diabetics in our study may merit further investigation of the relationship between copper and non-insulin dependent diabetes mellitus. [63]

2.3.5 Copper, Zinc, Manganese, and Magnesium Status and Complications of Diabetes Mellitus:

To evaluate copper, zinc, manganese, magnesium, and other indices of peroxidative status in diabetic and nondiabetic human subjects.

Convenience sample of 57 insulin-dependent or non-insulin-dependent diabetic subjects recruited from the diabetes clinic of the University of California, Davis, Medical Center and 28 nondiabetic subjects recruited from the staffs of the Departments of Internal Medicine and Nutrition. Individuals conducting laboratory analyses were blind to subject group. A fasting blood sample was collected from all subjects and appropriately processed for future analyses. A 24-h urine collection was obtained in a subset of subjects.

Hyperzincuria and hypermagnesuria were evident in diabetic subjects compared with control subjects. There were no differences in plasma magnesium or whole-blood manganese between groups. Plasma copper was higher and plasma zinc was lower in diabetic than in control subjects. When data were viewed with respect to specific diabetes-associated complications, diabetic subjects with retinopathy, hypertension, or microvascular disease had higher plasma copper concentrations compared with both diabetic subjects without complications and with control subjects. There were no significant differences between control and diabetic

subjects in erythrocyte copper-zinc superoxide dismutase activity or whole-blood glutathione peroxidase or glutathione reductase activities. Plasma peroxide concentrations were higher in diabetic than control subjects.

Diabetes can alter copper, zinc, magnesium, and lipid peroxidation status. Perturbations in mineral metabolism are more pronounced in diabetic populations with specific complications. It is not known whether differences in trace element status are a consequence of diabetes, or alternatively, whether they contribute to the expression of the disease. [64]

2.3.6 Metals in the pathogenesis of type 2 diabetes:

Minerals are one of the components of food, though they are not synthesized in the body but they are essential for optimal health. Several essential metals are required for the proper functioning of many enzymes, transcriptional factors and proteins important in various biochemical pathways. For example Zn, Mg and Mn are cofactors of hundreds of enzymes, and Zn is involved in the synthesis and secretion of insulin from the pancreatic beta-cells. Similarly, Cr enhances the insulin receptor activity on target tissues, especially in muscle cells. Insulin is the key hormone required to maintain the blood glucose level in normal range. In case of insulin deficiency or resistance, blood glucose concentration exceeds the upper limit of the normal range of 126 mg/dl. Persistent increase of blood serum glucose level leads to overt chronic hyperglycemia, which is a major clinical symptom of diabetes mellitus. Poor glycemic control and diabetes alters the levels of essential trace elements such as Zn, Mg, Mn, Cr, Fe etc. by increasing urinary excretion and their concomitant decrease in the blood. Hence, the main purpose of this review is to discuss the important roles of essential trace elements in normal homeostasis and physiological functioning. Moreover, perturbation of essential trace elements is also discussed in perspective of type 2 diabetes pathobiology. [61]

2.3.7 Selenium, Zinc and Copper in Plasma of patients with Type 1 Diabetes Mellitus in Different Metabolic Control States:

The Studies of selenium (Se), zinc (Zn) and copper (Cu) levels in diabetic patients have led to contradictory findings as to the possible relationship between the degree of diabetic control and the changes in mineral contents. In the present study the plasma Cu, Se and Zn contents of diabetic patients and healthy people were measured and the relationship between these contents and diabetic metabolic control, as determined by glycosylated hemoglobin (HbA_{1c}), was studied.

The mean plasma Se content in diabetic patients was significantly lower than in controls ($p<0.01$) and a negative correlation between the plasma contents of Se and HbA_{1c} was found. No statistically significant differences in plasma Zn contents, either between patients with type 1 diabetes mellitus and controls, or between patients with type 1 diabetes mellitus but different degrees of metabolic control, were found. A statistically significant sex difference in plasma Cu contents was observed in the control population. In females, statistically significant differences were found in plasma Cu contents between the control subjects and the diabetic patients with medium or poor metabolic control, as well as between diabetic patients with good and poor metabolic control. In males, the only statistically significant differences were between the control subjects and diabetic patients with poor metabolic control. The correlation between plasma contents of Cu and HbA_{1c} is not significant. [65]

2.3.8 Trace Elements in Diabetes Mellitus:

Diabetes Mellitus is the commonest major metabolic disease and most prevalent diseases worldwide.Its related morbidity is due to its micro and macro angiopathic complications.

The aim of this study was to measure and compare the serum levels of zinc and magnesium in normal individuals and in diabetic patients.

metabolism of several micronutrients in diabetic individuals. Zinc is one of the essential micronutrients of which status and metabolism is altered in this condition. This work is a short review about the close relation among zinc, glucose metabolism, and insulin physiology, as well as about the few experimental data about zinc absorption and zinc supplementation in diabetes mellitus patients. [69]

2.3.12 Zinc, Copper, Iron, and Chromium Concentrations in Young Patients with Type 2 Diabetes Mellitus:

Homeostasis of trace elements can be disrupted by diabetes mellitus. On the other hand, disturbance in trace element status in diabetes mellitus may contribute to the insulin resistance and development of diabetic complications. The aim of present study was to compare the concentration of essential trace elements, zinc, copper, iron, and chromium in serum of patients who have type 2 diabetes mellitus ($n = 20$) with those of nondiabetic control subjects ($n = 20$). The serum concentrations of zinc, copper, iron, and chromium were measured by means of an atomic absorption spectrophotometer (Shimadzu AA 670, Kyoto, Japan) after acid digestion. The results of this study showed that the mean values of zinc, copper, and chromium were significantly lower in the serum of patients with diabetes as compared to the control subjects ($P < 0.05$). Our results show that deficiency of some essential trace elements may play a role in the development of diabetes mellitus. [70]

2.3.13 Relationship between the Concentrations of Heavy Metals and Bioelements in Aging Men with Metabolic Syndrome:

Heavy metals may exacerbate metabolic syndrome (MS) but abnormal serum concentrations of bioelements may also co-exist with MS. The primary aim of the study was to assess the relationship of blood heavy metal and bioelement concentrations and MS, in men aged 50–75 years. Heavy metals—lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), tungsten (W), Macroelements—magnesium (Mg) and calcium (Ca), and microelements—iron (Fe), zinc (Zn)

this area is essentially unexplored in adequate clinical trials, which are worth being performed. [67]

2.3.10 Trace elements status in diabetes mellitus type 2: Possible role of the interaction between molybdenum and copper in the progress of typical complications:

It is well established that both, the deficiency and possible overload of mineral micronutrients have adverse health effects. It is also generally accepted that non-essential xenobiotics contribute to oxidative damage, which is considered one of the principal factors in diabetes and its complications. The purpose of this work was to gain an insight on the global role of metal/metalloids in the progress of diabetes mellitus type 2. In such approach, aluminum, vanadium, chromium, manganese, cobalt, nickel, copper, zinc, arsenic, selenium, molybdenum, mercury, cadmium and lead were determined by inductively coupled plasma-mass spectrometry (ICP-MS) in serum and urine of 76 diabetic patients (age 52 ± 8 years, 5–16 years of DM2, 52 subjects with slight-to-moderate complications and 24 with severe complications). A series of anthropometric and clinical parameters usually evaluated in the follow-up of patients were assessed by standard methods. Statistical analysis (unpaired *t*-test, analysis of correlation and principal component analysis) was then carried out in search of possible relationships existing among metals/metalloids and these parameters. The results obtained suggest that antagonistic interaction between molybdenum and copper might be involved in the progress of diabetes complications. [68]

2.3.11 Zinc and diabetes mellitus:

Diabetes mellitus is a group of metabolic disorders, the incidence of which varies widely throughout the world. The treatment of diabetes mellitus includes insulin, oral antidiabetic agents, and dietary regimens. Although the emphasis is on macronutrients intakes, there is strong evidence that there is an abnormal

Analysis of minerals was done in plasma by using a Varian Spectra AA 220 model atomic absorption spectrophotometer.

Our observations showed a definite lowering of serum magnesium ($p<0.001$) and serum zinc levels ($p<0.001$) were significant in diabetic group.

The cause of diabetic hypomagnesaemia is multifactorial. An altered metabolism, a poor glycaemic control and osmotic diuresis may be contributory factors. Decreased serum zinc levels in diabetes may be caused by an increase in urinary loss. These decreased levels of trace elements cause disturbances in glucose transport across cell membrane lead to insufficient formation and divretion of insulin by pancreas which compromise in the antioxidant defense mechanisms.[66]

2.3.9 Trace elements in glucometabolic disorders:

Many trace elements, among which metals, are indispensable for proper functioning of a myriad of biochemical reactions, more particularly as enzyme cofactors. This is particularly true for the vast set of processes involved in regulation of glucose homeostasis, being it in glucose metabolism itself or in hormonal control, especially insulin. The role and importance of trace elements such as chromium, zinc, selenium, lithium and vanadium are much less evident and subjected to chronic debate. This review updates our actual knowledge concerning these five trace elements. A careful survey of the literature shows that while theoretical postulates from some key roles of these elements had led to real hopes for therapy of insulin resistance and diabetes, the limited experience based on available data indicates that beneficial effects and use of most of them are subjected to caution, given the narrow window between safe and unsafe doses. Clear therapeutic benefit in these pathologies is presently doubtful but some data indicate that these metals may have a clinical interest in patients presenting deficiencies in individual metal levels. The same holds true for an association of some trace elements such as chromium or zinc with oral antidiabetics. However,

Consequently an atomic absorption spectrometer needs the following three components: a light source; a sample cell to produce gaseous atoms; and a means of measuring the specific light absorbed.

Reference value:

Zn: 0.5 – 1.2 mg/L

Cu: 0.7 – 1.4 mg/L

Chapter three

Materials & Methodology

Chapter Three

Materials and methods

3.1 Study design:

Descriptive cross sectional case control study during the period between January 2015 and June 2017.

3.2 Study area:

The study was conducted in River Nile State (Eddamer town which is located 300 km north to capital Khartoum).

3.3 Study population and Sample size:

Venous blood sample were obtained from (182) known diabetic patient, and (60) healthy individual as control group.

3.4 Specimens:

Each patient came in the morning (8-9) after 8 - 10 hours overnight fast. Then Five ml of venous blood was collected by standard procedure from the patients and divided in to 2ml whole blood with EDTA for HA1c and 3ml serum for (creatinine, fasting blood glucose, Cholesterol, Triglyceride and trace elements) and random urine sample for ACR.

3.5 Inclusion criteria:

Patients with diabetes mellitus type I and type II for case group and healthy people (non diabetic patients) for control group.

3.6 Exclusion criteria:

Any patient with other disease which had effect on parameters under study.

3.7 Ethical consideration:

This research was performed after the consent of all the people involved in it, and was taking into account the trust and strict confidentiality with respect to patients and information about them, and was scheduled, based on reliable

information and reliable source. This study was identical for all human rights. During data collection from patients or relatives, verbal consent was obtained, and names and personal data were completely secured and transferred to codes to keep patients' identities private.

3.8 Tools of data collection:

Interview questionnaire was applied and filled, which include name, age, sex, duration of DM, regular exercise, BMI and investigations (serum creatinine, HA1c, ACR, fasting blood glucose, Cholesterol, Triglyceride, Cu, Fe, Mg and Zn).

3.9 Data analysis:

The collected data was analyzed with statistical package for the social sciences (spss) version *(11.5*) Soft ware computer program, to obtained correlation; coefficient. Also independent T- test and Chi square test was used for calculating degree of variation, P.value <0.05 is considered to be significant.

3.10 Material required:

Syringe seals, plain container, alcohol swab, cotton, marker pens, centrifuge, automatic pipettes, test tubes, Spectrophotometer and Atomic absorption spectrometry (AAS).

3.11 Methodology (see appendix)

- Methodology for creatinine measurements.
- Methodology for cholesterol measurements.
- Methodology for triglyceride measurements.
- Methodology for blood glucose measurements.
- Methodology for magnesium measurements.
- Methodology for ferric measurements.
- Methodology for ACR measurements.

- Methodology for HA1c measurements: by ichroma
- Methodology for Zn and Cu measurements.

$$\frac{\text{urine albumin mg/dl}}{\text{Urine creatinine mmol/dl}}$$

3.12 Atomic absorption spectrometry (AAS): model 210 VGB:

Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram (μg dm–3) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another, higher, energy level.

How it works:

Atoms of different elements absorb characteristic wavelengths of light. Analysing a sample to see if it contains a particular element means using light from that element. For example with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms from the sample. In *(AAS)*, the sample is atomised – *ie* converted into ground state free atoms in the vapour state – and a beam of electromagnetic radiation emitted from excited lead atoms is passed through the vaporized sample. Some of the radiation is absorbed by the lead atoms in the sample. The greater the number of atoms there is in the vapour, the more radiation is absorbed. The amount of light absorbed is proportional to the number of lead atoms. A calibration curve is constructed by running several samples of known lead concentration under the same conditions as the unknown. The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the lead concentration in the unknown sample.

Table (4. 15): Demographical Data and some statistics

(A)

Gender		Exercise			Residence		Smoking	
Male	**Female**	**Regular**	**Irregular**	**Never**	**City**	**Rural**	**Yes**	**No**
40.7	59.3	9.9	26.4	63.7	57.1	42.9	12.1	87.9

(B)

Family history		Duration of DM / years				Complications	
Yes	**No**	**> 1**	**1 - 5**	**6 - 10**	**< 10**	**Yes**	**No**
73.6	26.4	15.4	50.5	22.0	12.1	61.5	38.5

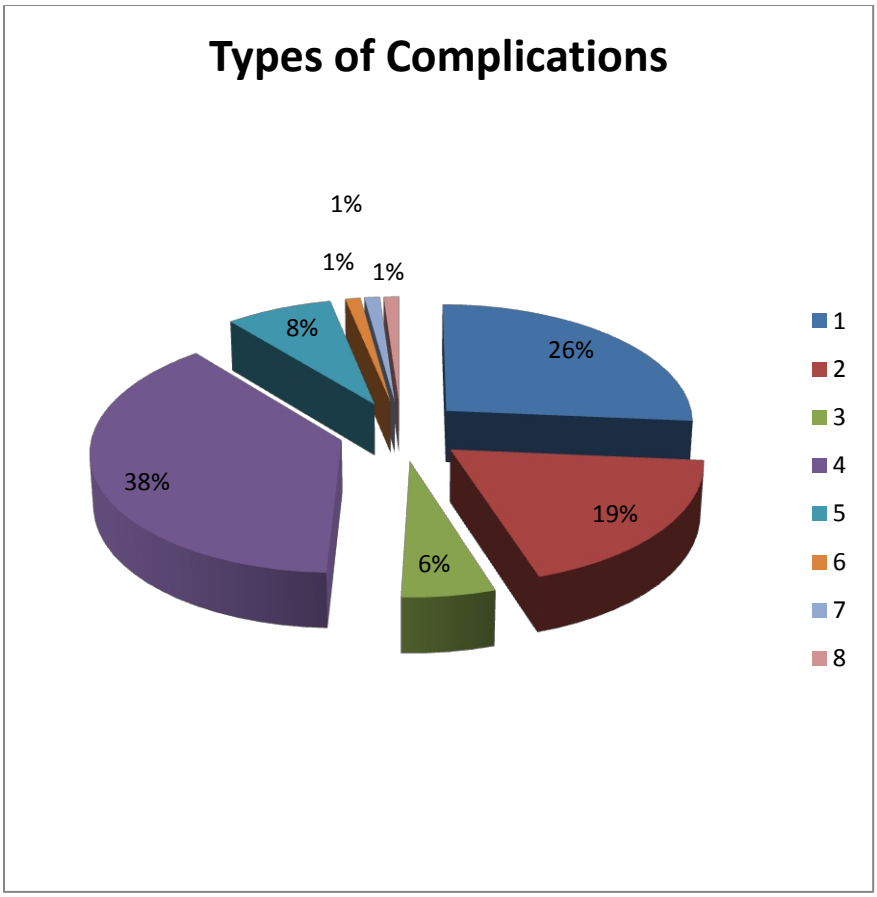


Figure (4) type of complications:

1/Hypertension 2/ Retinopathy 3/Nephropathy 4/No 5/ Retinopathy with Hypertension 6/ Nephropathy with Hypertension 7/Retinopathy, Nephropathy and Hypertension 8/Nephropathy with Retinopathy.

Chapter four

Results

Chapter Four

4.1 Results

Table (4 .1): The mean and Standard Deviation of zinc in non DM and DM patients:

Trace element		Mean ± Standard Deviation	P.value
Zn	Case	0.16 ± 0.05	0.000
	Control	0.60 ± 0.15	

Table (4 .2): The mean and Standard Deviation of copper in non DM and DM patients:

Trace element		Mean ± Standard Deviation	P.value
Cu	Case	0.18 ± 0.06	0.000
	Control	0.70 ± 0.13	

Independent t.test, level of significant ≤ 0.05

Table (4 .3): M and Standard Deviation of ferric in non DM and DM patients:

Trace element		Mean ± Standard Deviation	P.value
Fe^{+3}	Case	69.8 ± 35.0	0.00
	Control	99.0 ± 19.2	

Independent t.test, level of significant ≤ 0.05

Table (4 .4): The mean and Standard Deviation of magnesium in non diabetic and DM patients:

Trace element		Mean ± Standard Deviation	P.value
Mg^{+2}	Case	1.8 ± 0.29	0.001
	Control	2.0 ± 0.16	

Independent t.test, level of significant ≤ 0.05

Table (4. 5): The mean and Standard Deviation of ACR in non diabetic and DM patients:

Subject		Mean ± Standard Deviation	P.value
ACR	Case	3.34 ± 0.94	0.00
	Control	1.97 ± 0.22	

Independent t.test, level of significant ≤ 0.05

Table (4 .6): The mean of trace elements and its association with waist line, HbA1c, FBG, BMI, cholesterol, Triglyceride ACR, and creatinine in DM patients:

Subjects	P.value/ Pearson Correlation			
	Zn	Cu	Mg	Fe
HbA1c	0.21	0.01/-0.19	0.23	0.62
Waist line	0.002/-0.22	0.67	0.00l/-.027	0.00/+0.34
FBG	0.93	0.05/-0.20	0.66	0.99
BMI	0.37	0.38	0.01/-0.19	0.54
Cholesterol	0.09	0.35	0.10	0.58
Triglyceride	0.72	0.14	0.01/-0.19	0.21
Creatinine	0.28	0.00/+0.45	0.00/-0.25	0.001/+0.36
ACR	0.00/-0.35	0.00/-0.37	0.124	0.371

Correlation, level of significant less than or equal 0.05 and Pearson Correlation

Table (4 .7): Effect of duration of DM on trace elements levels:

Duration /years (100%)		Zn	Cu	Mg	Fe
< 1	(15.4%)	0.159	0.190	1.86	70.4
1-5	(50.5%)	0.168	0.185	1.81	70.7
6-10	(22.0%)	0.159	0.179	1.84	70.8
>10	(12.1%)	0.162	0.171	1.846	62.9
P.value		0.139	0.002	0.00	0.00

Table (4. 8): The mean and Standard Deviation of HbA1c in DM patients with deferent duration:

Duration /years (100%)		Mean of HbA1c ± Standard Deviation	P.value
< 1	(15.4%)	9.2 ± 2.52	0.00
1-5	(50.5%)	9.7 ± 2.49	
6-10	(22.0%)	9.4 ± 2.20	
>10	(12.1%)	10.6 ± 2.38	

Table (4 .9): Effect of Gender on trace elements levels in DM patients:

Gender (100%)	Zn	Cu	Mg	Fe
Male (40.7%)	0.166	0.180	1.91	82.0
Female (59.3%)	0.163	0.185	1.77	61.2
P.value	0.001	0.05	0.015	0.00

Table (4 .10): Effect of Smoking on trace elements levels in DM patients:

Smoking (100%)	Zn	Cu	Mg	Fe
Yes (12.1%)	0.171	0.174	1.85	99.0
No (87.9%)	0.163	0.184	1.83	65.7
P.value	0.035	0.007	0.75	0.000

Table (4 .11): The association of presence of Complications with trace elements levels in DM patients:

Complications (100%)	Zn	Cu	Mg	Fe
Yes (61.5 %)	0.162	0.190	1.84	72.4
No (38.5 %)	0.168	0.171	1.80	65.3
P.value	0.41	0.047	0.055	0.00

Table (4 .12): The association of Residence with trace elements levels in DM patients:

Residence (100%)	Zn	Cu	Mg	Fe
Urban (57.1 %)	0.172	0.182	1.82	70.1
Rural (42.9 %)	0.154	0.185	1.84	69.3
P.value	0.006	0.00	0.28	0.00

Table (4 .13): Effect of type of DM treatments on trace elements levels:

Medication (100%)	Zn	Cu	Mg	Fe
Pills (81.3 %)	0.166	0.179	1.87	66.7
Insulin (18.7 %)	0.155	0.20	1.67	82.9
P.value	0.093	0.00	0.00	0.00

Table (4. 14): The mean and Standard Deviation of HbA1c in type 1 and type II DM patients:

Medication (100%)	Mean of Hba1c ± Standard Deviation	P.value
Pills (81.3 %)	**9.61 ± 2.36**	0.00
Insulin (18.7 %)	**9.95 ± 2.80**	

Chapter five

Discssion
Conclusion
Recommendations

Chapter Five

5 –1: Discussion:

This present study was the first to be conducted in River Nile State during the period (January 2015 to June 2017), giving a first hand to handle and to bridge a gap in deficient informations concerning this topic in this state, aiming at the same time to determine the level of serum trace elements in *DM patients*, comparing them with a healthy control group, measuring their possible statistical correlations with glycemic control and some complications.

The study was including (242) individuals. (182) were diabetic patients (case study group), (74) of the case group accounted for (40.7%) were males, while (108) of the case group representing (59.3%) were females. (60) of the total population were healthy persons indicating the control group,

This study revealed a decrease in levels of serum trace elements (*Zn, Cu, Mg, and F*e) in *DM* patients when compared to control group (non Diabetic patients).

The results of this study were consistent with (Praveena & PaSula et al, 2013), indicating that: lowering of (serum *Mg*, P< 0.001) and (serum *Zn* < P.001) were significant in diabetic group. Then a comparison was done with the study of (Basaki,Saeb et al,2012) who found that: the mean values of (*Zn*), (*Cu*), and (*Cr*) were significantly lower in the serum of *DM* patients as compared to control subjects, but contradicting the study of (Zarger, shah et al, 1998) demonstrating that: (*Zn*) and (*Mg*) levels were not altered in *DM*, but the increased levels of (*Cu*) found in *DM*. On the other hand, study of (Zaki, Afridi et al 2008) denoted a high mean values of (*Cu*) and (*Fe*) detected in *DM* blood versus nondiabetic subjects. Focusing on a study done by (Waler, Uru-Hare et al, 1991) results showed higher plasma (*Cu*) and lower plasma (*Zn*) in *DM* than control subjects the results of this study partially contrasted regarding (*Cu)* and similar regarding (*Zn*).

This research prevailed that there were a negative correlation between (*Cu*) level and *HbA1c* and there was no effect of glycemic control on (*Zn*), (*Mg*) and (*Fe*) levels, These findings were partially similar to the study of (Ekmekcioglu, Prohaska et al, 2001) with exception to (*Cu*) results (which is contrast), these findings reflected that: trace elements concentrations were not dependant on the degree of glucose control as determined by correlation analysis between HBA_{1C} versus metal. Hereinafter, the results were not consistent with the study of (Khan and Awan, 2014), in which a conclusion expressing that: poor glycemic control and *DM* alters the levels of essential trace elements such as (*Zn*), (*Mg*), (*Mn*), (*Cr*), and (*Fe*).

This recent study revealed that there was no effect of *DM* type on (*Zn*) level, whereas the (*Cu*) and (*Fe*) levels were lower in *DM type II* than *DM type I* while (*Mg*) was lower in *DM type I DM* patients compared to *type II*. These findings contradicted the results obtained by (Zarger, Shah et al, 1998), who detected that the plasma levels of (Zn) were significantly higher in *type I DM* patients, whereas the levels of plasma (Cu) and (Mg) were not significantly altered.

Moreover, the study indicated that: there was a significance correlation between (*Cu*) &(*Fe*) levels and duration of *DM*, while the level of (*Cu*) was reduced with increased duration of *DM*, but the level of (*Fe*) was observed to be decreased in a group of higher DM duration (*more than 10 years*). These results contradicted the results obtained by Zarger's contributers whom arrived at that: duration of *DM* did not influence (*Cu*), (*Zn*), or (*Mg*) conentrations.

Regarding a relation between *DM* complications and trace elements, the study results contributed that: there was a linkage between mean of (*Cu*) & (*Fe*) among *DM* patients complications, Moreover, higher levels of (*Cu*) & (*Fe*) were appeared to be associated with the presence of complications among *DM subject*s. On the other hand, there were no complications. These findings were similar to the

conclusions obtained by walter and his team whom interpreted that: diabetic subjects with retinopathy, hypertension, or microvascular disease had higher plasma (*Cu*) concentrations.

The results findings of the present study had shown that: (*Zn*), (*Mg*), and (*Fe*) were found to be higher in males than females while (*Cu*) was lower in males. These results were in contrast to (Zarger) results who attributed that: sex did not influence (*Cu*), (*Zn*), or (*M*g) concentrations.

This study was carried out trying to bridge some informational gaps due to inavailabilty of local literature, to correlate the results with these already published, to find the differences and interrelationships, Some findings were associated with biochemical & nutritional parameters were sumarized as follows:

negative association between (*Mg*) and body mass index and this finding was similar to study of Rotter, Kosik-Bogacka whoconcluded that: negative correlations Mg-BMI), Positive correlation between (*Cu*) and creatinine and Negative relationship between (*Cu*) & (*FBG*) and this result contradicts to study of of Evliyaoğlu, Kebapçılar who attributed that: positive correlation was found between serum Cu and glucose ($r=502$, $p<0,05$) levels.

5.2 Conclusion:

- Serum zinc, copper, magnesium and Ferric were lower in case group (Diabetic patients) than control group (healthy people).
- The study showed there was negative correlation between *(Cu)* and *HbA1c* and no effect glucose control to *(Zn), (Mg) and (Fe).*
- Negative correlation between (*Mg* with body mass index and between *(Cu and FBG)* and Positive correlation between (creatinine and *Cu*).
- Cu and Fe were higher in *type I DM* than *type II DM* while Mg was lower in *type I of DM* than *type II DM.*
- The level of *(Cu)* was reduced with increase duration of *DM* and low level of *(Fe)* found in group of higher duration (more than 10 years).

5 – 3: Recommendations:

- Health education programs would apply for diabetic patients so as to be aware of trace elements and its importance.
- Further studies about trace elements and its association with some diabetic complications.
- Good Control of *DM* to avoid disturbance of serum trace elements and diabetes complications.
- Regular assessment of trace elements for *DM* to reduce the severity and some complications of diabetes mellitus.

Chapter Six

Referances
Appendix

6.1 References

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