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Determination the Value of Chromium, Zinc, Phosphorus and Manganese in Patients with Type 2 Diabetes Mellitus by Different Technique

Thesis

Submitted to the Council of College of Medicine, University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master in Science / Clinical Biochemistry

By

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بسم الله الرحمن الرحيم

وَٱلرَّٰسِخُونَ فِى ٱلْعِلْمِ يَقُولُون آمَنَّابِهِ كُلٌّ مِّنْ عِندِ رَبِّنَا

صدق الله العلي العظيم

سورة ال عمران جزء من الآية ٧

Supervisor Certification

We certify that this thesis entitled "**Determination the value of chromium, zinc, phosphorus and manganese in patients with type 2 diabetes mellitus by different technique** " Was carried under our supervision at the College of Medicine, University of Babylon, as a partial fulfillment of the requirements for the degree of Master of Science in Clinical Biochemistry.

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In view of the available recommendations, I forward this thesis for debate by the examination Committee.

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Date / / 2022

College of Medicine/ University of Babylon

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Dedication

I dedicate this project to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on His wings only have I soared.

I also dedicate this work to my husband, who has encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I have started. To my daughters, who have been affected in every way possible by this quest.

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Afaf Abdul - Kadhim Hussein

Summary

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone. A relationship was observed between diabetes mellitus and tracer elements in many research studies. In many cases, an alteration in the metabolism of these minerals was demonstrated. Insulin action was reported to be potentiated by some trace elements like chromium, zinc, manganese and phosphorus. Proposed mechanisms of enhancement of insulin action by trace elements include activation of insulin receptor sites, serving as cofactors or components for enzyme systems which are involved in glucose metabolism, increasing insulin sensitivity and acting as antioxidants for preventing tissue peroxidation.

The aim of the study to estimate the levels of serum zinc, phosphorus, chromium and manganese in diabetes mellitus type 2 patients and compare them with control group and evaluation the impact of these levels on the severity of the disease, and determine the correlation between them.

The study was conducted on 180 subjects attending Al- Suwayrah hospital between Octobers to December 2021. Subjects had age range within 35-69 years. This study is case-control study with sample size (180), include (90) T2DM patients (56 female and 34 male) with normal renal function and (90) apparently healthy control group, sample size was calculated by sample size equation from the community health department in the college.

There were significant differences between means of age and body mass index according to study group ($P<0.001$, P value ≤ 0.05 was significant).

Significant differences between means of blood urea, serum creatinine and fasting blood glucose according to study group (P value for urea 0.038*, f or creatinine 0.006* and FBG <0.001, P value ≤ 0.05 was significant).

Significant differences between means of Zn (spectrophotometric) according to study group (P value <0.001). Zn in this study measured in two methods spectrophotometric and atomic absorption, there were significant differences between means of Zn between these two techniques among diabetic patients (p value<0.001). Also, significant differences between means of Zn (atomic absorption) according to study group (p value<0.001, P value ≤ 0.05 was significant). ROC curve for the sensitivity and specificity of Zn / Sp (µg/dl) in diabetes mellitus, (Cut-off point was ≤ 109.50 (µg/dl)), AUC=0.71, P <0.001*, 95% CI (0.634-0.787), the sensitivity was 80.0%, the specificity was 61.1%, positive predictive value was 67.28%, negative predictive value was 75.34% and overall accuracy was 70.55%. ROC curve for the sensitivity and specificity of Zn / atom (µg/dl) in diabetes mellitus, (Cut-off point was ≤ 75.40 (µg/dl)), AUC=1.00, P <0.001*, 95% CI (1.000-1.000), the sensitivity was 100.0%, the specificity was 100.0%, positive predictive value was 100.0%, negative predictive value was 100.0% and overall accuracy was 100.0%.

Significant differences between means of Ch and Mn (atomic absorption) according to study group (p value <0.001, P value ≤ 0.05 was significant**)**. There were no significant differences between means of phosphate (spectrophotometric) (P value = 0.305).

The study concludes a larger link between BMI and the onset of diabetes. The study concludes that population with diabetes have low Zn level compared to healthy population. Scientific evidences highlighted in this review point out changes in zinc metabolism which contributes to an oxidative stress manifestation in patients with type 2 diabetes mellitus.

Low level of serum Mn in diabetic group in this study. Diabetes was more common in people who had low blood Mn levels, suggesting that Mn may have a role in glucose regulation. When compared to nondiabetic healthy control subjects found that type 2 diabetics had reduced serum Ch levels. As early in the development of diabetes plasma phosphate levels may be normal or even low, these deregulations may be difficult to distinguish. Depending on the results obtained, the atomic absorption technique is more sensitive, specific and superior for trace elements determination.

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List of Abbreviations

Abbreviation	Full name
AI	Adequate intake
ASCVD	Arteriosclerotic Cardiovascular disease
ATP	Adenosine triphosphate
AUC	Area Under the Curve
BMI	Body-mass index

CDC	Centres for Disease Control
CI	Confidence Interval
CKD	Chronic kidney disease
CP	Creatine phosphate
CrPic	Chromium picolinate
CVD	Cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DKA	Diabetic ketoacidosis
DKD	Diabetic kidney disease
DMT1	Divalent Metal Transporter 1
DNA	Deoxyribonucleic acid
DRI	Dietary Reference Intakes
ESRD	End-Stage Renal Disease
FBG	Fasting blood glucose
FFA	Free fatty acid
FGF-23	Fibroblast growth factor 23
FNB	Food and Nutrition Board
GDM	Gestational diabetes mellitus
Hb A1C	Haemoglobin A1c
HOMA-IR	Homeostatic model assessment for insulin resistance
IDF	International Diabetes Federation
IR	Insulin resistance
LDL-C	Low density lipoprotein cholesterol
MnSOD	Manganese superoxide dismutase
MTs	Metallothioneins
NB	New born
NGSP	National Glycohemoglobin Standardization Program
NHANES III	National Health and Nutrition Examination Survey
NPT2	Neomycin phosphotransferase 2
P	Probare
PCOS	Polycystic ovarian syndrome
PEP	Phosphoenol pyruvate
PH	Power of hydrogen
RDA	Recommended dietary allowance
RNA	Ribonucleic acid
ROC	Reactive oxygen species
RSH	Represents of functional group (thiol group)
SOD	Superoxide dismutase
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes
WHO	World Health Organization

Chapter One

Introduction

and

Literature Review

1.Introduction

1.1. Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone [1]. Low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities. The severity of symptoms is due to the type and duration of diabetes [2]. Some of the diabetes patients are asymptomatic especially those with type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetes may lead to stupor, coma and if not treated death, due to ketoacidosis or rare from non ketotic hyperosmolar syndrome [3].

A relationship was observed between diabetes mellitus and tracer elements in many research studies [4,5]. In many cases, an alteration in the metabolism of these minerals was demonstrated. Insulin action was reported to be potentiated by some trace elements like chromium, zinc, manganese and phosphorus. Proposed mechanisms of enhancement of insulin action by trace elements include activation of insulin receptor sites, serving as cofactors or components for enzyme systems which are involved in glucose metabolism, increasing insulin sensitivity and acting as antioxidants for preventing tissue peroxidation [5].

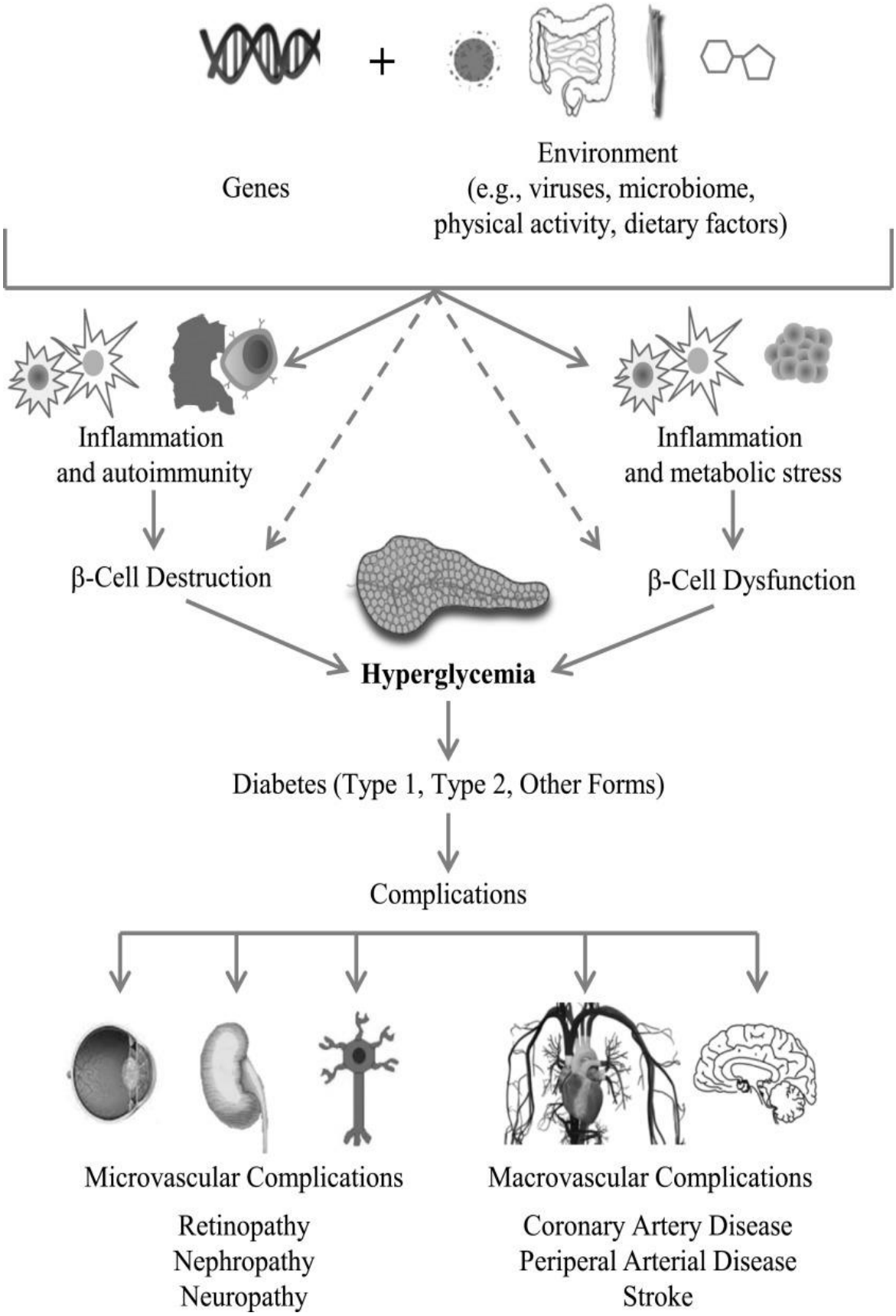

Figure 1-1 The Necessary Common Factor to all forms of Diabetes [2].

1.1.2.Epidemiology:

Diabetes affects at least 246 million people globally, according to the World Health Organization (WHO). This number is expected to reach 380 million by 2025, according to the International Diabetic Federation. Diabetes mellitus is found all across the world, however it is more prevalent (particularly type 2) in developed countries. However, the biggest increase in prevalence is projected in Asia and Africa, where the majority of patients will most likely be found by 2030 [6].

The rise in diabetes cases in developing countries coincides with the trend of urbanization and lifestyle changes, most notably a "Western-style" diet. According to the National Center for Chronic Disease Prevention and Health Promotion (Centers for Disease Control and Prevention (CDC), one out of every three Americans born after the year 2000 will get diabetes throughout their lifetime.

According to the National Health and Nutrition Examination Survey (NHANES III) in the United States, 18-20% of people over 65 years old have diabetes, with 40% having diabetes or its precursor, impaired glucose tolerance. Diabetes is one of the top ten, if not the top five, most important diseases in the developed world, and it is growing in importance there and abroad [7].

1.1.3.Classification:

The majority of diabetes patients can be divided into three categories: type 1, type 2, and gestational diabetes [8].

1.1.3.1.Type 1 Diabetes Mellitus:

Type 1 diabetes mellitus (T1DM), commonly known as childhood diabetes or juvenile diabetes, is characterized by the loss of insulin-producing beta cells in the islets of Langerhans in the pancreas, resulting in

insulin insufficiency. This form of diabetes is further divided into immune-mediated and idiopathic diabetes.

The bulk of T1DM is immune-mediated, with beta cell loss being the result of a T-cell-mediated autoimmune onslaught. T1DM, which accounts for about 5-10% of diabetes mellitus cases in North America and Europe, has no known preventive measures[9]. When onset begins, the majority of those affected are otherwise healthy and of a healthy weight. Even in the early stages of type 1 diabetes, insulin replacement remains the primary treatment. Ketosis and diabetic ketoacidosis (DKA) can develop without insulin, resulting in coma or death.

Insulin sensitivity and responsiveness are frequently normal, especially in the early stages of the disease. T1DM can affect both children and adults, although it is commonly referred to as "juvenile diabetes" because it accounts for the majority of diabetes cases in children. T1DM cannot be reversed or prevented by diet or exercise [10].

1.1.3.2.Type 2 Diabetes Mellitus:

Type 2 Diabetes Mellitus (T2DM) is one of the most common metabolic disorders worldwide and its development is primarily caused by a combination of two main factors: defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to insulin [11].

Insulin release and action have to precisely meet the metabolic demand; hence, the molecular mechanisms involved in the synthesis and release of insulin, as well as the insulin response in tissues must be tightly regulated. Therefore, defects in any of the mechanisms involved can lead to a metabolic imbalance that leads to the pathogenesis of T2DM.

Over 90% of diabetes mellitus cases are T2DM, a condition marked by deficient insulin secretion by pancreatic islet β-cells, tissue insulin

resistance (IR) and an inadequate compensatory insulin secretory response[12].

Progression of the disease makes insulin secretion unable to maintain glucose homeostasis, producing hyperglycemia. Patients with T2DM are mostly characterized by being obese or having a higher body fat percentage, distributed predominantly in the abdominal region. In this condition, adipose tissue promotes IR through various inflammatory mechanisms, including increased free fatty acid (FFA) release and adipokine deregulation. The main drivers of the T2DM epidemic are the global rise in obesity, sedentary lifestyles, high caloric diets and population aging, which have quadrupled the incidence and prevalence of T2DM [13].

The organs involved in T2DM development include the pancreas (β-cells and α-cells), liver, skeletal muscle, kidneys, brain, small intestine, and adipose tissue. Evolving data suggest a role for adipokine dysregulation, inflammation, and abnormalities in gut microbiota, immune dysregulation, and inflammation have emerged as important pathophysiological factors [14].

1.1.3.3.Gestational Diabetes:

Gestational diabetes mellitus (GDM) is a common pregnancy complication, in which spontaneous hyperglycemia develops during pregnancy [15]. According to the most recent (2017) International Diabetes Federation (IDF) estimates, GDM affects approximately 14% of pregnancies worldwide, representing approximately 18 million births annually [16].

Risk factors include overweight/obesity, westernized diet and micronutrient deficiencies, advanced maternal age, and a family history of insulin resistance and/or diabetes. While GDM usually resolves following delivery, it can have long-lasting health consequences, including increased

risk for type 2 diabetes (T2DM) and cardiovascular disease (CVD) in the mother, and future obesity, CVD, T2DM, and/or GDM in the child.

This contributes to a vicious intergenerational cycle of obesity and diabetes that impacts the health of the population as a whole. Unfortunately, there is currently no widely-accepted treatment or prevention strategy for GDM, except lifestyle intervention (diet and exercise) and occasionally insulin therapy—which is only of limited effectiveness due to the insulin resistance that is often present. While emerging oral antidiabetics, such as glyburide and metformin, are promising, concerns remain about their long-term safety for the mother and the child [17].

Therefore, safe, effective, and easy-to-administer new treatments are sought. In order to develop such treatments, a thorough understanding of the pathophysiology of GDM is required[18].

1.1.3.4. Specific types of diabetes due to other causes:

E.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as in the treatment of HIV/AIDS or after organ transplantation)[19]

1.1.3.2.1.Etiology and Rick Factors:

Risk Factors and Pathophysiology T2DM risk factors include a complex combination of genetic, metabolic and environmental factors that interact with one another contributing to its prevalence. Although individual predisposition to T2DM due to non-modifiable risk factors (ethnicity and family history/genetic predisposition) has a strong genetic basis, evidence from epidemiological studies suggests that many cases of T2DM can be prevented by improving the main modifiable risk factors (obesity, low physical activity and an unhealthy diet) [20].

A. Ethnicity and Family History/Genetic Predisposition.

Globally, the incidence and prevalence of T2DM are found to vary widely depending on ethnicity and geographical region with Japanese, Hispanics and Native Americans having the highest risks. It has been shown higher incidence rates in Asians compared with a White American population [21], and white population in the UK, where the highest risk is among the black population.

Whilst no clear reasons have been found, contributing factors such as modern lifestyle factors (which promote obesity), socioeconomic and direct genetic propensity or gene environmental interactions have been postulated. Genetic predisposition plays an important part in the risk of developing T2DM.

Over the past decade, several T2DM genome-wide association studies have shown the complex polygenic nature of T2DM in which most of these loci increase T2DM risk through primary effects on insulin secretion, and a minority act through reducing insulin action [22]. Dimas et al. grouped these variants on the basis of their potential intermediate mechanisms in T2DM pathophysiology, with four variants fitting a clear IR pattern; two reducing insulin secretion with fasting hyperglycemia; nine lowering insulin secretion with normal fasting glycemia; and one altering insulin processing [23].

According to these data, the genetic architecture of T2DM is highly polygenic, and additional association studies are needed to identify most T2DM loci. Interactions between susceptibility loci and environmental factors could underlie the missing heritability of T2DM thus the impact of a given genetic variant can be modulated by the environmental factors (and vice versa) as evidenced by both observational studies and clinical trials [24].

B. Obesity, Low Physical Activity and Unhealthy Diet.

Obesity (body-mass index [BMI]≥30 kg/m2) is the strongest risk factor for T2DM and is associated with metabolic abnormalities resulting in IR. There exists an inverse linear relationship between BMI and the age at diagnosis of T2DM [25].

The exact mechanisms by which obesity induces T2DM and IR remain to be elucidated; however, numerous factors have shown a significant role in the development of this pathological process, which involves both cell-autonomous mechanisms and inter-organ communications.

A sedentary lifestyle is another risk factor for T2DM as shown by the Women's Health Study and in the Kuopio Ischemic Heart Disease Risk Factor Study, which showed a reduction of 34% and 56% reduction of developing T2DM in participants walking 2–3 h a week or at least 40 min a week, respectively [26]. There are three primary benefits of physical activity on the delay of T2DM onset. First, the contraction of skeletal muscle cells induces an increase in blood flow into the muscle, enhancing glucose uptake from plasma. Second, physical activity reduces the notorious intra-abdominal fat, which is a known risk factor that promotes IR. Finally, moderate-intensity exercise has been shown to improve glucose uptake by 40%. Physical activity improves glucose uptake and insulin sensitivity but it can also improve or even reverse inflammation and oxidative stress, which are T2DM predisposing factors [27].

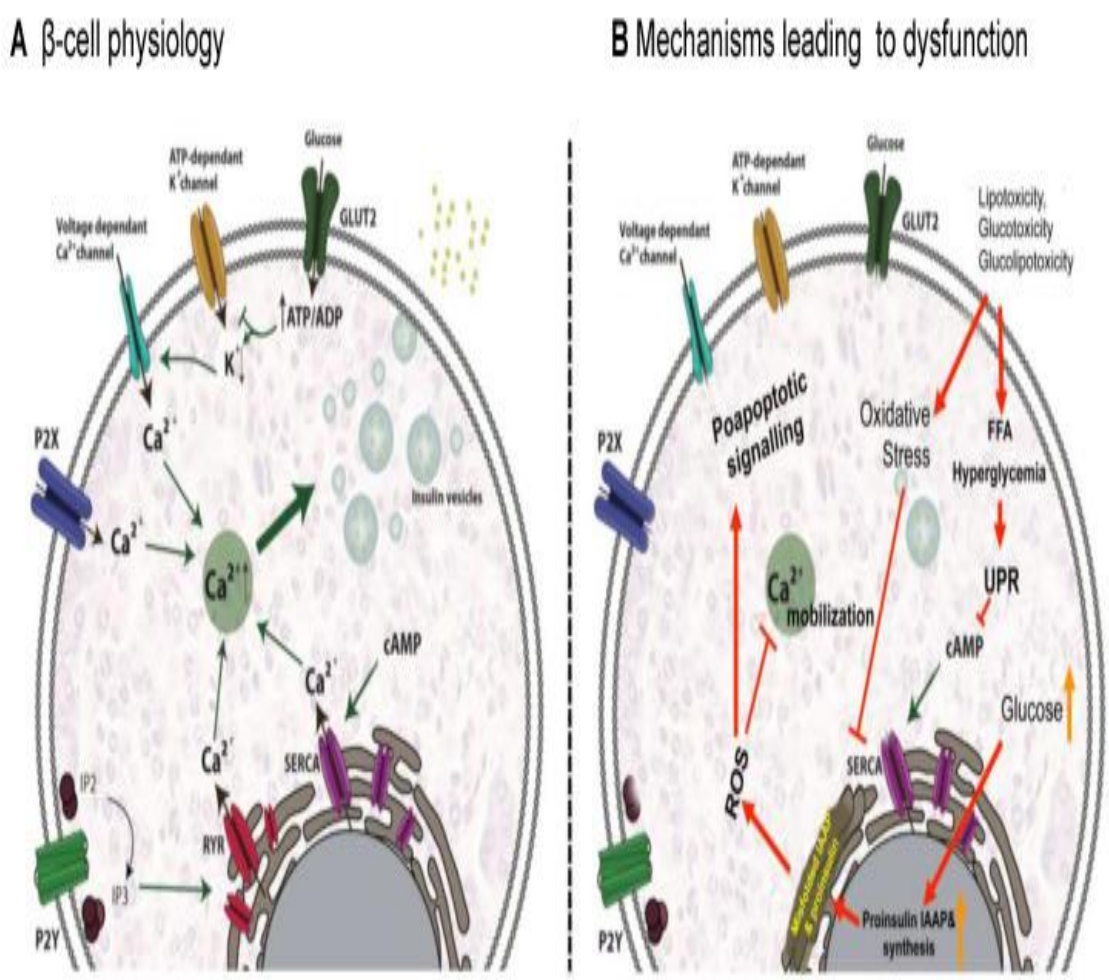

Figure 1-2. Signaling pathways involved in insulin secretion in β-cells in physiological conditions (**A**) and mechanisms leading to dysfunction (**B**)[1].

C. Pathophysiology.

Regarding the pathophysiology of the disease, a malfunctioning of the feedback loops between insulin action and insulin secretion results in abnormally high glucose levels in blood [28]. In the case of β-cell dysfunction, insulin secretion is reduced, limiting the body's capacity to maintain physiological glucose levels. On the other hand, IR contributes to increased glucose production in the liver and decreased glucose uptake both in the muscle, liver and adipose tissue. Even if both processes take place early in the pathogenesis and contribute to the development of the disease,

β-cell dysfunction is usually more severe than IR. However, when both β-cell dysfunction and IR are present, hyperglycemia is amplified leading to the progression of T2DM [29].

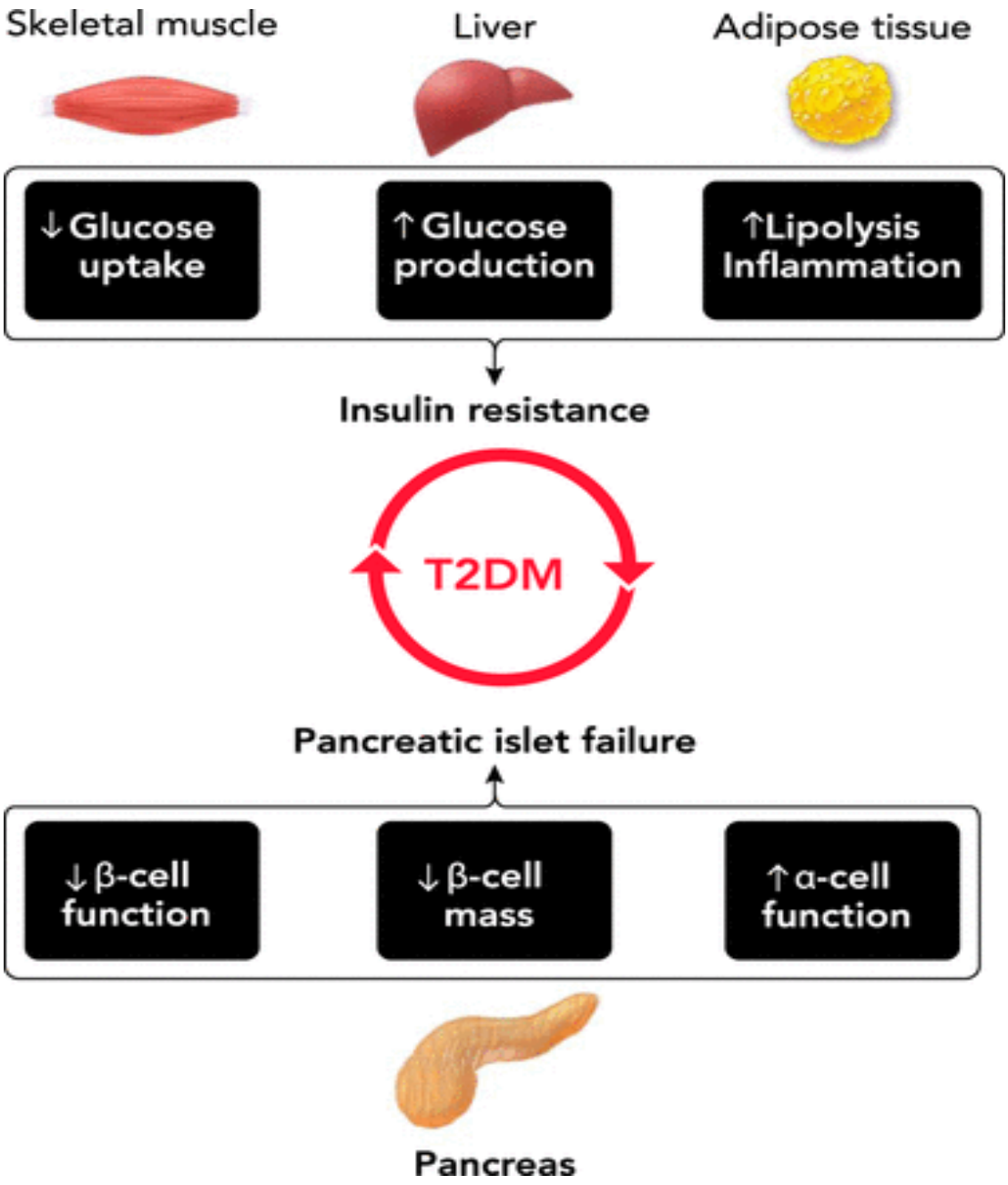

Figure 1-3. Pathophysiology of Type 2 diabetes mellitus [1].

1.1.3.2.2. Hyperglycemia:

Hyperglycemia is derived from the Greek words hyper (high) + glykys (sweet/sugar) + haima (blood sugar) (blood). Hyperglycemia is defined as blood glucose levels of more than 125 mg/dL while fasting and more than 180 mg/dL two hours after eating. With a fasting plasma glucose of 100 mg/dL to 125 mg/dL, a patient has impaired glucose tolerance, or pre-diabetes. A fasting blood glucose level of more than 125 mg/dL qualifies a patient as diabetic [29].

When hyperglycemia is left untreated, it can cause damage to the eye, kidneys, nerves, heart, and peripheral vascular system, among other major life-threatening consequences. To avoid illness complications and enhance patient outcomes, it is critical to treat hyperglycemia properly and efficiently. Reduced insulin secretion, decreased glucose utilization, and increased glucose production are all factors that contribute to hyperglycemia. The equilibrium between hepatic glucose synthesis and peripheral glucose uptake and utilization is known as glucose homeostasis. Insulin is the most essential glucose homeostasis regulator [30].

The following are some of the secondary causes of hyperglycemia:[31]

1.Endocrine disorders that produce peripheral insulin resistance, such as Cushing syndrome, acromegaly, and pheochromocytoma.

2.Destruction of the pancreas due to chronic pancreatitis, hemochromatosis, pancreatic cancer, and cystic fibrosis.

4.Gestational diabetes affects about 4% of all pregnancies and is caused by a decrease in insulin sensitivity.

5.Dextrose infusion and total parental nutrition.

6.Reactive, as observed in patients who have had surgery or who are seriously unwell.

The following are some of hyperglycemia's major risk factors:[31]

1. Native Americans, Hispanics, Asian Americans, Pacific Islanders, or African Americans who weight more than 120 percent of their ideal body weight.
2. Family history of type 2 diabetes.
3. History of gestational diabetes.
4. Hyperlipidemia or hypertension.
5. polycystic ovarian syndrome (PCOS).

1.1.3.2.3. Hypoglycemia:

A plasma glucose concentration of less than 70 mg/dL is commonly used to diagnose hypoglycemia; however, signs and symptoms may not appear until plasma glucose concentrations fall below 55 mg/dL. Since 1938, the Whipple's triad symptoms have been used to describe hypoglycemia. The practitioner must first notice hypoglycemia symptoms, then get low blood glucose, and then demonstrate quick alleviation of symptoms by correcting the low blood glucose with glucose treatment, according to Whipple's triad.

Under normal physiologic settings, glucose is the brain's major metabolic fuel. The brain, unlike other human tissues, has an extremely limited supply of glucose. For proper metabolic activity, the brain requires a continuous supply of blood glucose [32]. A disruption in the glucose supply may result in problems. As a result, the body has evolved preventive systems to guard against low serum blood glucose (hypoglycemia).

Gluconeogenesis and glycogenolysis in the liver keep serum glucose levels stable during fasting periods. Gluconeogenesis is the process of producing glucose from non-carbohydrate sources. Protein, lipids, pyruvate, and lactate are examples of non-carbohydrate sources.

Glycogenolysis, on the other hand, is the breakdown of glycogen into glucose compounds. Hepatocytes (liver cells) and myocytes (muscle cells) perform the majority of glycogenolysis. Hypoglycemia is most commonly encountered in diabetic individuals who are receiving pharmacologic treatment. When getting therapy, people with type 1 diabetes are three times more likely than patients with type 2 diabetes to have hypoglycemia [33].

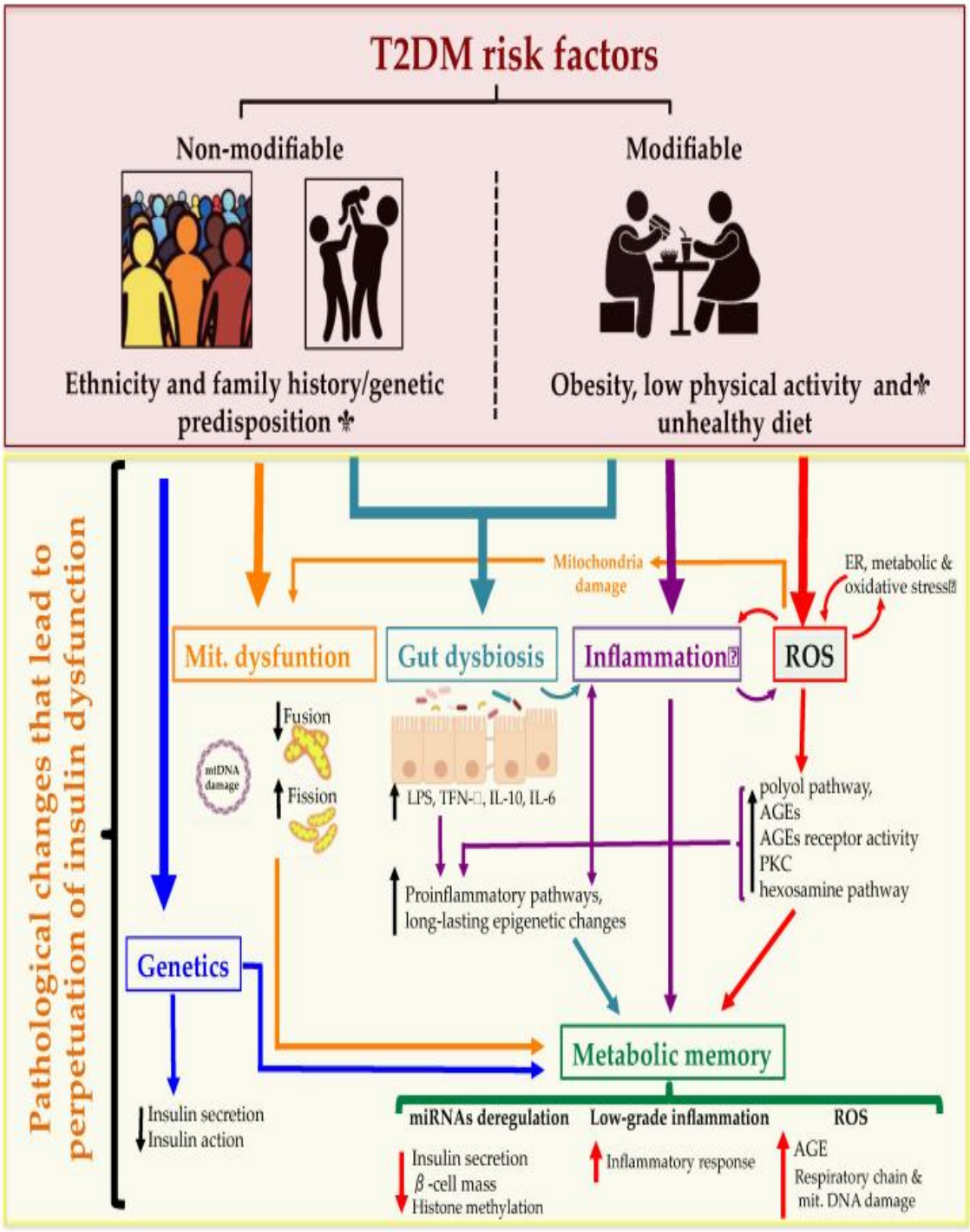

Figure 1-4. Type 2 Diabetes Mellitus (T2DM) risk factors and the pathological changes leading to the perpetuation of insulin dysfunction [1].

1.1.3.2.4. Complications:

Regardless of the specific type of diabetes, complications involve microvascular, macrovascular, and neuropathic issues. Microvascular and macrovascular complications vary according to the degree and the duration of poorly control diabetes and include nephropathy, retinopathy, neuropathy, and ASCVD events, especially if it is associated with other comorbidities like dyslipidemia and hypertension[34].

One of the most devastating consequences of DM is its effect on cardiovascular disease (ASCVD). Approximately two-thirds of those with DM will die from a myocardial infarction or stroke. In T2DM, fasting glucose of more than 100 mg/dL significantly contributes to the risk of ASCVD, and cardiovascular risk can develop before frank hyperglycemia [35].

DM is also a common cause of blindness in adults aged 20 to 74 years in the United States. Diabetic retinopathy contributes to 12000 to 24000 new cases of blindness annually, and treatments generally consist of laser surgery and glucose control.

Renal disease is another significant cause of morbidity and mortality in DM patients. It is the leading contributor to end-stage renal disease (ESRD) in the United States, and many patients with ESRD will need to start dialysis or receive a kidney transplant. If the albuminuria persists in the range of 30 to 300 mg/day (microalbuminuria), it seems to be a predictable earliest marker for the onset of diabetic nephropathy. Once macroalbuminuria (greater than 300 mg/24 hr) sets in, the progression to ESRD hastens up. The random spot urine specimen for measurement of the albumin-to-creatinine ratio is a quick, easy, predictable method that is the most widely used and preferred method to detect microalbuminuria. Two of three tests, done over a six-month showing a persistent level greater than 30 mcg/mg creatinine, confirms the diagnosis of microalbuminuria.

DM is also the leading cause of limb amputations in the United States; this is primarily due to vasculopathy and neuropathy associated with DM. Many patients who develop neuropathy need to have regular foot exams to prevent infection from wounds that go unnoticed [36].

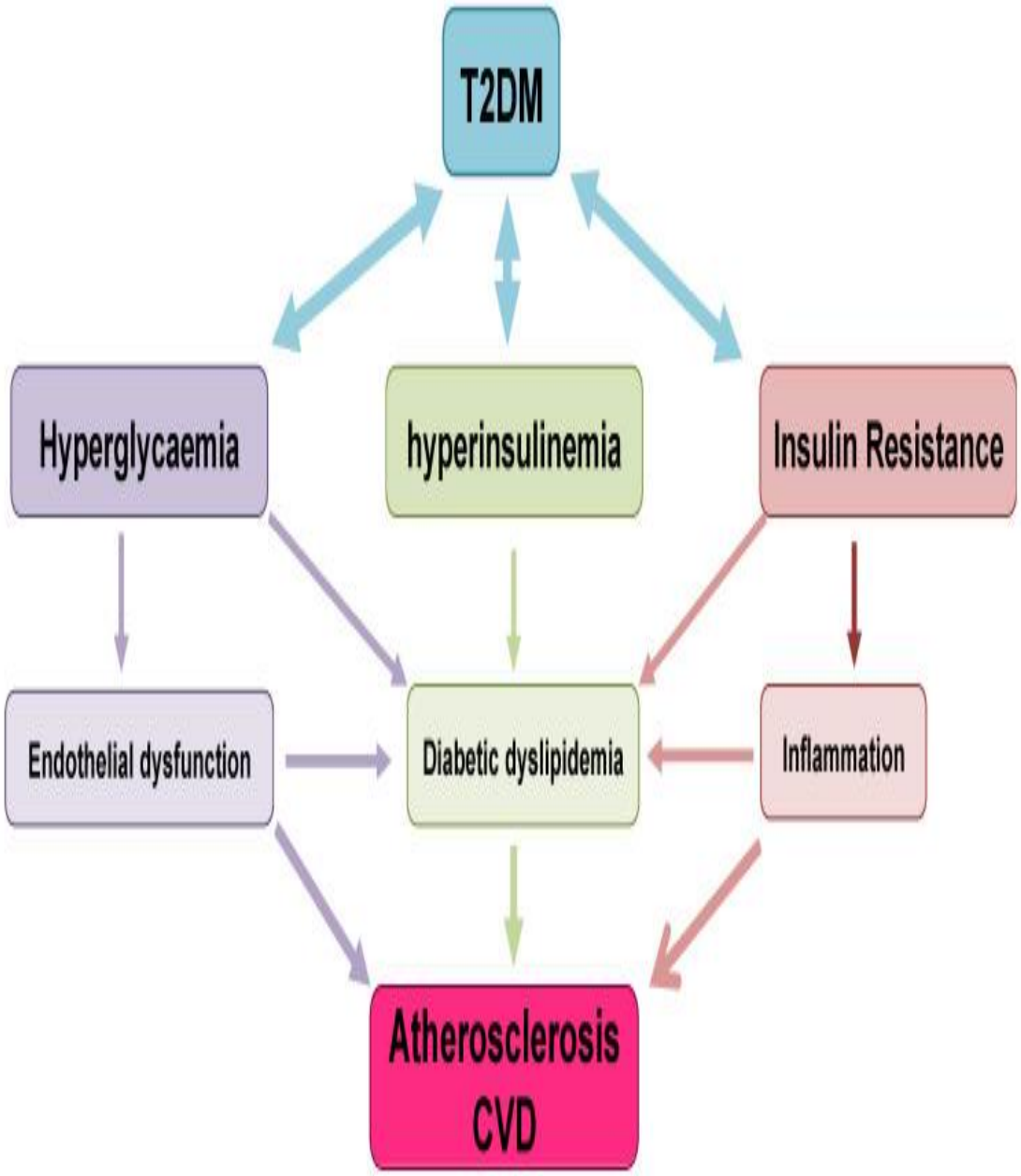

Figure 1-5. Factors implicated in cardiovascular risk outcomes from T2DM and the interactions between them. T2DM derived hyperglycemia, hyperinsulinemia and IR causes endothelial dysfunction, diabetic dyslipidemia and inflammation leading to CVD. The flowchart illustrates the multiple interactions among the implicated factors [1].

1.1.3.2.5. Diagnosis:

A. Signs and Symptoms:

The signs and symptoms of diabetes are disregarded by many because of the chronic progression of the disease. People do not consider this as a serious problem because unlike many other diseases the consequences of hyperglycemia are not manifested immediately. People are not aware that damage can start several years before symptoms become noticeable. This is unfortunate because recognition of early symptoms can help to get the disease under control immediately and to prevent vascular complications. Considering the asymptomatic nature of type 2 diabetes in the early stages, it is essential that the people are educated on its warning signs [37].

The Warning signs and symptoms of diabetes mellitus type2 are:[38]

1. Unexplained weight loss.
2. Frequent fatigue.
3. Irritability.
4. Repeated infections especially in the: genital areas, urinary tract, skin, oral cavity, delayed wound healing.
5. Dry mouth.
6. Burning, pain, numbness on feet.
7. Itching.
8. Reactive hypoglycemia.
9. Acanthoses nigricans-the presence of velvety dark patches of the neck, arm pit, groin which is an indicator of insulin.
10. decreased vision.
11. Impotence or erectile dysfunction.

The classic symptoms of diabetes such as polyuria, polydipsia and polyphagia occur commonly in type 1 diabetes, which has a rapid

development of severe hyperglycemia and also in type 2 diabetes with very high levels of hyperglycemia. Severe weight loss is common only in type 1 diabetes or if type 2 diabetes remains undetected for a long period. Unexplained weight loss, fatigue and restlessness and body pain are also common signs of undetected diabetes. Symptoms that are mild or have gradual development could also remain unnoticed [39].

B. Diagnostic criteria:

The hemoglobin A1C criterion or the plasma glucose concentration can both be used to diagnose diabetes (fasting or 2-hour plasma glucose). Glucose in the Plasma After a Fast (FPG).

B.1. Fasting Plasa Glucose (FPG):

After an overnight fast of 8 hours, a blood sample is collected. According to the American Diabetes Association, a fasting plasma glucose (FPG) level of higher than 126 mg/dL (7.0 mm/L) is indicative of diabetes [40].

B.2. Oral Glucose Tolerance Test for Two Hours (OGTT):

The plasma glucose level is tested before and two hours after ingesting 75 grams of glucose in this test. If the plasma glucose (PG) level in a 2-hour sample is higher than normal, diabetes is diagnosed. a concentration of less than 200 mg/dL (11.1 mmol/L) It is a routine test as well, although it is inconvenient and more expensive than FPG, and it has significant variability difficulties. For 3 to 5 days, patients must eat a diet containing at least 150 grams of carbohydrates per day and avoid taking any medications that can affect glucose tolerance, such as steroids and thiazide diuretics [41].

B.3. A1C for Glycated Hemoglobin (Hb):

This test offers you an average of your blood glucose levels over the previous two to three months. Diabetic patients have a Hb A1C level of more than 6.5 percent (48 mmol/mol). Hb A1C is a simple, quick, and standardized test that exhibits reduced variation due to pre-analytical factors. Acute illness or stress have little effect on it [42].

Hb A1C is expensive and has a number of drawbacks, including reduced sensitivity, as detailed below. The National Glycohemoglobin Standardization Program (NGSP) accredited technique standardized to the Diabetes Control and Complications Trial (DCCT) test should be used to determine Hb A1C. Sickle cell illness, pregnancy, hemodialysis, blood loss or transfusion, and erythropoietin medication are all factors that alter it [43]. It hasn't been thoroughly tested in non-white groups.

Anemia caused by a lack of iron or vitamin B12 causes a false increase in Hb A1C, restricting its use in areas where anemia is common. In addition, the relationship between Hb A1C and FPG is unsatisfactory in youngsters and the elderly. If the person is asymptomatic, all of the aforementioned tests should be performed later to confirm a diagnosis of diabetes mellitus.

Random plasma glucose levels of more than 200 mg/dL are also adequate to diagnosis DM in patients with characteristic hyperglycemia symptoms (increased thirst, hunger, and urination) FPG, 2-hour PG during 75-g GTT, and Hb A1C are all acceptable tests for diagnosing diabetes. There is no correlation between the outcomes of these tests [44].

1.2. Chromium:

Two oxidation states of chromium are considered to be biologically and environmentally relevant based on their stability in the presence of water and oxygen. Compounds containing chromium (6 +) are mutagenic and carcinogenic when inhaled and potentially when ingested orally in large quantity as well [73].

1.2.1. Dietary Sources:

Chromium is ubiquitous in foods at very low concentrations. Most dietary chromium apparently is derived from processing of food with stainless steel equipment; thus, humans probably evolved on a diet containing significantly less chromium than the current diets of people of developed nations [74].

1.2.2. Requirements:

In 2001, the Institute of Medicine determined that insufficient evidence existed to set an Estimated Average Requirement for chromium. Consequently, Adequate Intakes of 35 and 25 μg/d for young men and women, respectively, were set based on estimated mean intakes. More recently, in 2014 the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority determined that "there is no evidence of beneficial effects associated with chromium intake in healthy subjects" and that "the setting of an Adequate Intake for chromium is also not appropriate" [75].

1.2.3. Reference Intervals:

Reference intervals (RIs) are widely used in the process of making medical diagnoses, therapeutic management decisions, and other physiological assessments. Serum chromium level normally is 0.62-0.97 mg/dl or 26.92 nanomoles/L (nmol/L) [76].

1.2.4. Absorption and Excretion:

Chromium is absorbed together with other metal ions in the gut through the unsaturated passive transport. The efficiency of this process is very low with the average absorption ranging from 0.4 – 2.5% [77]. The absorption process depends on the Cr content in the diet and on the chemical form of this element and other food components. Studies conducted in rats showed increased absorption of Cr used in the form of nicotinate (1.3%) and picolinate (1.1%) in comparison to chromium chloride (0.9%). It was shown that absorption of Cr in humans in the form of chromium chloride is much lower (0.1–0.4%) than of chromium picolinate (2.8%) or chromium given as the yeast chromium (5–10%).

Organic sources of Cr (i.e., picolinate, or propionate-methionine salt) are much better absorbed than inorganic forms (e.g., oxides), and lead to the increase of these compounds' concentration in tissues [78]. The highest dose-accumulation correlation of Cr in the tissues is observed after administration of Cr nanoparticles. However, other factors present in the diet show a significant impact on the amounts of Cr absorbed from the gastrointestinal tract. Starch, simple sugars, ascorbic acid, oxalic acid, nicotinic acid, some amino acids, aspirin increase absorption of this element [79], while high concentrations of phosphate, calcium, magnesium, titanium, zinc, vanadium and iron reduce the rate of this process.

After absorption from the intestine, chromium (III) is released into the bloodstream where it is bound by proteins involved in iron metabolism. In vitro and in vivo studies in rats have shown that about 80% Cr in the blood is associated with transferrin. In this complex, Cr is transported to the cells, and the efficiency of Cr transfer through the cell membrane depends on insulin concentration [80]. Chromium is found in all animal

tissues and is present at the concentrations of several to tens of µg/kg, rarely exceeding 100 µg/kg. The highest concentrations are found in the liver, kidneys and spleen, while slightly lower levels are observed in heart, muscle, pancreas, lungs, bones and brain [81].

It has been shown that certain tissues, such as bones, testis and epididymis, are capable of storing Cr in a long-term manner, in comparison to heart, pancreas or brain, where the turnover of chromium ions is relatively short. More than 80% of Cr is removed from the body in the form of urine, while the remaining part of this element is excreted via faeces and sweat. In humans, consumption of large amounts of sugar, exhaustive physical exercise, pregnancy and lactation leads to increased Cr excretion in the urine. Negative Cr balance was observed in patients suffering from diabetes type 1 enhanced secretion of this element in the urine was also found in rats after intramuscular injection of insulin [82].

1.2.5. Role of Chromium in Biological System:

Chromium might play a role in carbohydrate, lipid, and protein metabolism by potentiating insulin action [83]. Although the precise mechanism for this activity has not been identified, scientists have proposed that chromium binds to an oligopeptide to form chromodulin, a low-molecular-weight, chromium-binding substance that binds to and activates the insulin receptor to promote insulin action [84]. Chromium might also have antioxidant effects [83].

In 2001, the Food and Nutrition Board (FNB) of the National Academies of Sciences, Engineering, and Medicine considered chromium to be an essential nutrient based on its effects on insulin action. However, recent research has suggested that although chromium might have benefits at pharmacologic amounts (e.g., in the hundreds of mcg), it is not an essential mineral because an absence or deficiency of chromium does not

produce abnormalities that can be reversed with the addition of chromium [85].

The FNB has not evaluated chromium since 2001. However, in 2014, the European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies concluded that no convincing evidence shows that chromium is an essential nutrient and, therefore, setting chromium intake recommendations would be inappropriate [84]. In the blood, most chromium is bound to plasma proteins, particularly transferrin, and only about 5% is unbound. Chromium accumulates mainly in the liver, spleen, soft tissue, and bone [86].

1.2.6. Role of Chromium in Diabetes Mellitus type 2:

Furthermore, it is imperative to identify some preventive strategies to decrease the risk of diabetes and its complications. Recently the dietary supplements including chromium were used to manage T2DM and improve glycemic control [87]. Chromium as an essential element is found in foods and dietary supplements that plays an important role in insulin function and glucose metabolism in mammalian [88]. There is evidence that in diabetic patients, subclinical chromium deficiency is associated with elevated blood glucose, insulin, and lipid levels that may adversely affect the management of diabetes and its repletion after experimental dietary depletion led to improvement in glycemic status.

Chromium with the mechanism that expands insulin cell signaling through the low molecular weight chromium-binding substance increases the sensitivity of insulin receptors in the plasma membrane and improves glycemic control. Some studies have shown that chromium could improve both glucose and insulin metabolism. however, some studies reported no beneficial effect for chromium in T2DM patients [89].

In 2020 Aria Tavakoli Talab, Hadi Abdollahzad, Seyyed Mustafa Nachvak, Yahya Pasdar, Shahryar Eghtesadi, Azimeh IzadiMir Amir Aghdashi, Mohammad Reza Mohammad Hossseini Azar, Sedighe Moradi, Behzad Mehaki, and Shima Moradi found that supplementation of 400 µg CrPic resulted in no improvement in body weight and BMI. However, CrPic could attenuate LDL-C, total cholesterol, and HOMA-IR significantly; while no significant was occurred in FBG levels. It seems that CrPic supplementation might help T2DM patients to improve the disease-associated disturbance of lipid profile and insulin resistance [90].

1.3. Zinc:

Zinc is a trace mineral that is only second to iron in terms of body content. Adult humans have 2 to 3 grams of zinc, but it's difficult to know how much zinc they have, especially when they're sick [45]. Zinc is required for the proper functioning of the immune system. Zinc is required for cell division, cell growth, wound healing, carbohydrate breakdown, insulin action enhancement, and the sense of smell and taste. Zinc is required for optimal growth and development during pregnancy, infancy, and childhood [46].

1.3.1. Dietary Sources:

Zinc is widely distributed in food mainly bound to proteins. The bioavailability of dietary zinc is dependent on the digestion of these proteins to release zinc and allow it to bind to peptides , amino acids, phosphate, and other ligands within the intestinal tract. The most available dietary sources of zinc are red meat and fish, whereas white meat and flesh from young animals provide less zinc. Wheat germ and whole bran are good sources [47].

1.3.2.Requirements:

The recommended dietary allowance (RDA) is the average daily level of intake that is sufficient to meet the nutrient requirements of nearly all (97 to 98%) healthy people. The adequate intake (AI) is the level established when there is not enough scientific research evidence to develop an RDA.

The following is the case for zinc in Table 1-1 and 1-2 :[48]

Table 1-1 :RDA of Infants and Children

Age	adequate intake (AI)
0 to 6 months	2 mg per day (mg/day).
7 to 12 months	3 mg/day
1 to 3 years	3 mg/day
4 to 8 years	5 mg/day
9 to 13 years	8 mg 8 mg/day
Supplementation Dose	5 to 5 to 20 mg/day

Table 1-2 :RDA of Adolescents and Adults

Groups	Age in years	adequate intake (AI**)**
Males	14 and > 14	11 mg/day
Females	14 to 18	9 mg/day
Females	19 and > 19	8 mg/day
Pregnant females	19 and > 19	11 mg/day
Lactating females	19 and > 19	12 mg/day

1.3.3.Reference intervals:

The reference range of serum zinc in the normal adult population, was found as(60-120) μg/dl [49].

1.3.4.Absorption and Excretion:

Zinc is absorbed in the small intestine by a carrier-mediated mechanism. Under normal physiologic conditions, transport processes of uptake are not saturated. The fraction of zinc absorbed is difficult to determine because zinc is also secreted into the gut. Zinc administered in aqueous solutions to fasting subjects is absorbed efficiently (60-70%), whereas absorption from solid diets is less efficient and varies depending on zinc content and diet composition. Generally, 33% is accepted as the average zinc absorption in humans [50].

More recent studies have suggested different absorption rates for different population groups based on their type of diet and phytate: Zinc molar ratio. Zinc absorption is concentration dependent and increases with increasing dietary zinc up to a maximum rate. In addition, zinc status may influence zinc absorption. Zinc-deprived humans absorb this element with increased efficiency, whereas humans on a high-zinc diet show a reduced efficiency of absorption.

About 70% of the zinc in circulation is bound to albumin, and any condition that alters serum albumin concentration can have a secondary effect on serum zinc levels. Although, serum zinc represents only 0.1% of the whole body zinc, the circulating zinc turns over rapidly to meet tissue needs [51].

Loss of zinc through gastrointestinal tract accounts for approximately half of all zinc eliminated from the body. Considerable amount of zinc is secreted through the biliary and intestinal secretions, but most of it is reabsorbed. This is an important process in the regulation of zinc balance.

Other routes of zinc excretion include urine and surface losses (desquamated skin, hair, sweat). Measurements in humans of endogenous intestinal zinc have primarily been made as fecal excretion; these indicate that amounts excreted are responsive to zinc intake, absorbed zinc and physiologic need [52].

1.3.5.Role of Zinc in Biological System:

Zinc performs its biochemical functions as a divalent cation primarily when bound to enzymes and other proteins. It is redox inert and has catalytic and regulatory roles in cellular biology. This metal is indispensable to the growth and development of microorganisms, plants, and animals. Zinc is essential as a catalytic, structural, and regulatory ion and is involved in homeostasis, immune responses, oxidative stress, apoptosis, and aging [53].

Of all the trace element metals found in humans, only iron is more abundant than zinc. Hence, if Hemoglobin-bound iron is not considered, zinc becomes the most abundant transition metal in the body. This element can be found in all body tissues and secretions in relatively high concentrations with 85 % of all of the body zinc found in muscle and bones, 11 % in the skin and liver, and the remainder in other tissues with the highest concentrations in the prostate and parts of the eye.

The total zinc content of plasma is usually approximately 100 g zinc/100 mL of plasma depending on (1) age, (2) pregnancy, (3) sex, and (4) time of day as the plasma zinc content is higher in the morning than in the afternoon. Zinc protecting biological structures from damage by free radicals may be due to several factors: an adequate level and maintenance of metallothioneins (MTs), an essential component of superoxide dismutase (SOD), a protective agent for thiols (RSH), thus preventing the interaction between chemical groups with iron to form free radicals [54].

1.3.6.Role of Zinc in Diabetes mellitus type 2 :

Chronic hyperglycemia statue in diabetes favors the manifestation of oxidative stress due to high production of reactive oxygen species and/or a decrease of the antioxidant defense system activity linked to lipid peroxidation and oxidative cellular injury themselves resulting in damages in the metabolism of lipids, proteins and DNA and from changes in cells functions[55].

Hormonal, biochemical and nutritional disorders present in type 2 diabetic individuals have been subject to researches with the aim of clarifying the mechanisms involved in the pathogenesis of this disease. Regarding both biochemical and nutritional disorders, studies show changes in the mineral metabolism and the activity of antioxidant enzymes such as zinc and superoxide dismutase[56]. Zinc plays a relevant role in antioxidant defense in patients with type 2 diabetes mellitus. This mineral may act by different protection mechanisms by notably being an essential cofactor for more than 300 enzymes, such as superoxide dismutase. This mineral also facilitates reduction and neutralization of free radicals. Considering changes in zinc metabolism and in superoxide dismutase enzyme activity present in type 2 diabetic patients simultaneously with the importance of these compounds in antioxidant defense[57].

In 2020 Dhedhi M. Farooq, Ali F. Alamri, Basmah K. Alwhahabi, Ashraf M. Metwally, and Khalid A. Kareem found Diabetic patients have Zn deficiency compared to normal individuals and poor glycemic control is associated with low Zn levels [58].

1.4. Phosphorus:

Phosphorus, an essential mineral, is naturally present in many foods and available as a dietary supplement. Phosphorus is a component of bones, teeth, DNA, and RNA. In the form of phospholipids, phosphorus is also a component of cell membrane structure and of the body's key energy source, ATP. Many proteins and sugars in the body are phosphorylated. In addition, phosphorus plays key roles in regulation of gene transcription, activation of enzymes, maintenance of normal pH in extracellular fluid, and intracellular energy storage. In humans, phosphorus makes up about 1 to 1.4% of fat-free mass. Of this amount, 85% is in bones and teeth, and the other 15% is distributed throughout the blood and soft tissues [59].

1.4.1.Dietary Sources:

Many different types of foods contain phosphorus, including dairy products, meats and poultry, fish, eggs, nuts, legumes, vegetables, and grains. In the United States, dairy products contribute about 20% of total phosphorus intakes, and bakery products (e.g., breads, tortillas, and sweet bakery products) contribute 10%. Vegetables and chicken contribute 5% each [60].

1.4.2. Requirements:

Table 1-3 : Recommended Dietary Allowances (RDAs) for phosphorus [61].

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	100 mg	100 mg		
7–12 months	275 mg	275 mg		
1–3 years	460 mg	460 mg		
4–8 years	500 mg	500 mg		
9–13 years	1,250 mg	1,250 mg		
14–18 years	1,250 mg	1,250 mg	1,250 mg	1,250 mg
19+ years	700 mg	700 mg	700 mg	700 mg

1.4.3. Reference Intervals:

Table 1-4 : Comments Reference Ranges Serum Phosphorus[61].

Age	Ranges (mg/dL)
NB	4.3-10.5
<1 mo	5.0-9.5
1-12 mo	4.8-8.1
1-2 yr	4.5-6.7
2-16 yr	4.0-5.7
16 yr+	2.9-4.7

1.4.4.Absorption and Excretion:

The absorption rate for the phosphorus naturally contained in food is 40%–70%; phosphorus from animal sources has a higher absorption rate than that from plants [62]. Calcium from foods and supplements can bind to some of the phosphorus in foods and prevent its absorption [63]. According to one analysis, a very high calcium intake of 2,500 mg/day binds 0.61–1.05 g phosphorus. In infants, phosphorus bioavailability ranges from 85%–90% for human milk to approximately 59% for soy-based formulas.

The absorption of phosphate in the intestinal epithelial cells occurs via a co-transport mechanism through active sodium/phosphate (Na^+/P_i) co-transporters, which involves at least three different types of Na^+/P_i, i.e., NPT2a, b and c. This mechanism can be inhibited by nicotinamide, so that the administration of niacin may be used as an effective approach to reduce the intestinal absorption of phosphorus and to lower circulating phosphorus level [64]. Upon intake of natural (non-enhanced) food, approximately 60% of the dietary phosphorus is absorbed in the intestine as phosphate. This proportion may increases to 80% when the circulating level of calcitriol increases. In normal adults, dietary phosphorus load is counterbalanced by a phosphorus excretion in urine that equals its net intestinal absorption. The so-called phosphatonines, in particular FGF-23 as described above, reduce the expression of co-transporters NPT2a, b and c but, but unlike PTH they also inhibits the renal 1-alpha hydroxylase leading to a reduction in the in-vivo synthesis of calcitriol by the kidneys [65].

Hence, the net phosphorus load from food intake is a function of phosphorus content of the ingested food, phosphorus bioavailability from different sources of diet, the food preparation modality and of vitamin D status.

urinary phosphorus excretion has been proposed as a recovery biomarker of total dietary phosphorus intake. An important assumption in the use of urinary phosphorus as a recovery biomarker is that dietary phosphorus intake is excreted at a constant rate in the urine for all individuals, regardless of dietary or nondietary factors [66].

1.4.5. Role of Phosphorus in Biological System:

Phosphates are essential to modern biological systems, and their wide and varied range of biological roles is a testament to their value in controlling chemistry and building robust structures in an aqueous environment. They provide the stable ligation required to fix information in RNA and DNA, contribute to cellular structure in phospholipids, serve as the basic currency of biochemical energy (e.g., ATP, phosphoenol pyruvate (PEP), creatine phosphate (CP)) and feature in a wide variety of metabolites and commonly observed post-translational protein modifications [67].

Westheimer provided a detailed analysis of the essential role of phosphate in living systems more than 30 years ago highlighting in particular, that phosphates are ionized at physiological pH, due to a low first pK_a (pK_a = 2.2, 7.2, 12.3). This ionic character renders phosphates hydrophilic, and facilitates their retention within a cell membrane. Importantly, in the case of RNA and DNA ligation, the ionic structure of phosphates allows the ligation of two nucleosides whilst retaining a negative charge at the phosphodiester. The charge carried by the phosphodiester ligations between nucleotides provides an essential solubilizing element, and importantly, protects the phosphodiesters from hydrolysis.

Beyond the structural role phosphates play in biology, they also serve a multitude of integral roles in energy metabolism, where again the

kinetic stability afforded by ionization is an essential element in exploiting phosphates. Kinetic stability and thermodynamic activation are coupled to excellent effect in phosphate moieties, to provide a robust chemical drive for biochemical transformations, whilst allowing enzymatic modulation of reactions, and regulation of metabolic pathways [68] .

1.4.6. Role of Phosphorus in Diabetes Mellitus type 2 :

Patients with type 2 diabetes mellitus (DM) are a high risk group and metabolic disorders contribute to the prediction of morbidity and mortality in this population. Metabolic disturbances including changes in serum calcium, magnesium or phosphate P can explain why dyslipidemia, hyperglycemia and hyperuricemia, which are related to obesity, impact the progression to type 2 DM. It has been reported that low serum levels of P are associated with increased insulin resistance in the healthy population. Moreover, a previous experimental study using rats suggested that P depletion results in low insulin secretion by pancreatic beta cells, due to high intracellular calcium and inhibition of adenosine triphosphatase production.

Thus, it has been suggested that low serum P may disturb the regulation of serum glucose in non-DM with obesity[69]. Phosphate is essential for life, as it participates in the structure of cellular membranes as a material of nucleic acids, phospholipids and adenosine triphosphate. Additionally, P plays a crucial role in cellular signaling through reactions of phosphorylation. Homeostasis of P is affected by multiple interactions between the intestine, parathyroid glands, kidneys and bone. Serum P levels are dependent on the absorption in the gut from dietary P, the excretion and reabsorption of P in the kidneys, and the movement of P between the extracellular and skeletal pools. Parathyroid hormone and fibroblast growth factor 23 play an important role in the regulation of

serum P by mediating urinary P removal. Elevated serum P is recognized as an independent predictor for advanced vascular disease in chronic kidney disease (CKD)[70].

However, epidemiological studies showed that all-cause mortality was independently related to increased serum P in all populations, even without CKD and serum P levels in the upper normal reference range [71].In 2020 Vaia D Raikou, Despina Kyriaki, and Sotiris Gavriil found High serum P contributes to vascular and metabolic disturbances in elderly patients with type 2 DM and renal impairment [72].

1.5.Manganes:

Manganese (Mn) is an environmentally abundant essential metal required for numerous indispensable biochemical processes throughout the human body. Its function arises secondary to its inclusion within protein structures as a cofactor. Without its presence, the human body's immune function, biochemical regulation of energy consumption, growth potential, coagulation, and hemostatic function, and mechanisms to remove byproducts of aberrant oxidative stress would be significantly diminished [91].

1.5.1. Dietary Sources:

Plant sources have much higher manganese concentrations than animal sources. For a thorough list of food sources and their manganese concentrations, see the review by Freeland-Graves et al. [92]. Whole grains (wheat germ, oats, and bran), rice, and nuts (hazelnuts, almonds, and pecans) contain the highest amounts of manganese. Chocolate, tea, mussels, clams, legumes, fruit, leafy vegetables (spinach), seeds (flax, sesame, pumpkin, sunflower, and pine nuts), and spices (chili powder, cloves, and saffron) are also rich in manganese [93].

1.5.2. Requirements:

The Institute of Medicine's DRI for manganese cites ~2 mg/day as an adequate intake for adults males 2.3 mg/day and for adults females 1.8 mg/day [94].

1.5.3. Reference Interval:

Manganese status is difficult to assess and not routinely measured in clinical practice. Normal serum concentrations of manganese range from 0.55-1.4μg/dl in serum [95] .

1.5.4. Absorption and Excretion:

Mn-containing food provides the major source of Mn intake in humans. The bioavailability of ingested Mn is about 3–5% in humans . Mn is absorbed from the intestine by either active transport or facilitated diffusion[96].While there is no specific Mn transporter identified in the gut, accumulating evidence has indicated that several iron transporters are involved in Mn absorption. The divalent metal transporter 1 (DMT1) plays an important role in intestinal uptake of Mn. DMT1.

The human body contains about 10 to 20 mg manganese, of which 25% to 40% is in bone. The liver, pancreas, kidney, and brain also contain manganese. The body maintains stable tissue manganese concentrations through regulatory control of manganese absorption and excretion .More than 90% of absorbed manganese is excreted via bile into the feces, and a small amount is reabsorbed [97].

1.5.5. Role of Manganese in Biological System:

Manganese is an essential trace element that is naturally present in many foods and available as a dietary supplement. Manganese is a cofactor for many enzymes, including manganese superoxide dismutase, arginase, and pyruvate carboxylase. Through the action of these enzymes, manganese is involved in amino acid, cholesterol, glucose, and carbohydrate metabolism; reactive oxygen species scavenging; bone formation; reproduction; and immune response. Manganese also plays a role in blood clotting and hemostasis in conjunction with vitamin K [98].

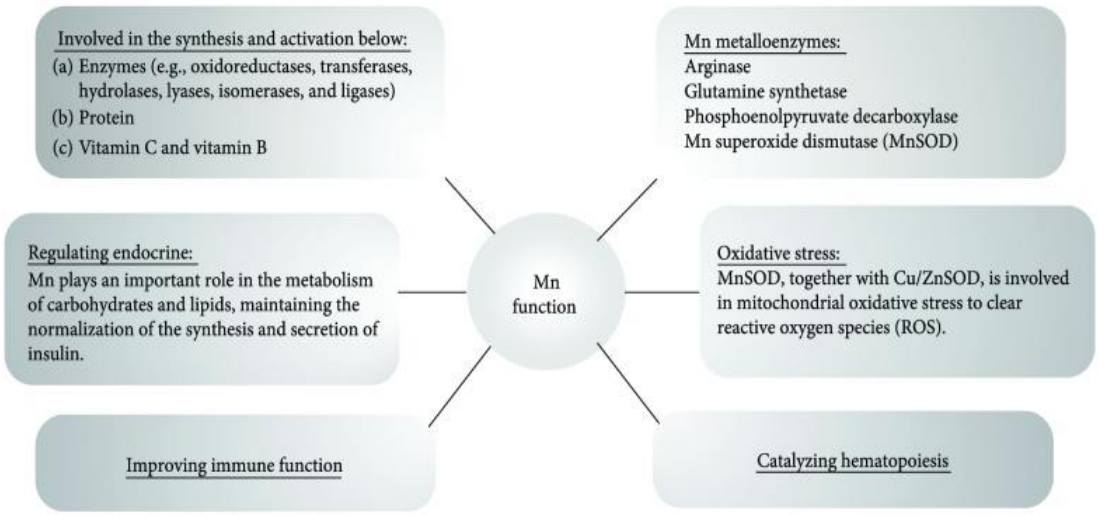

Figure 1-6 . Physiological roles of Mn[98].

1.5.6. Role of Manganese in Diabetes Mellitus type 2:

Mn is one of the essential micronutrients for humans and dietary consumption is a principal source of Mn in the body .Animal studies have found an association between dietary Mn and glucose metabolism: low dietary Mn can impair insulin secretion and glucose metabolism ,while Mn supplementation modifies the enzyme profiles of carbohydrate metabolism and improves high-fat-diet-induced beta cell injury and insulin resistance in animal models of diabetes. However, acute oral Mn supplementation does not consistently affect glucose tolerance in non-diabetic or type 2 diabetic individuals, and few studies have reported the possible effect of dietary Mn in type 2 diabetes.

The association between dietary Mn and type 2 diabetes in human studies is unclear, especially in longitudinal cohort studies. Plasma Mn and Mn superoxide dismutase (MnSOD) have been recommended as potential biomarkers for Mn nutritional status in the human body, and both of them have been associated with type 2 diabetes. Low serum Mn has been associated with a high risk of type 2 diabetes. However, higher levels of Mn in serum and urine have also been reported in individuals with diabetes, and similar whole-blood Mn levels between diabetic and non-diabetic individuals have been observed.

MnSOD plays a major role in the downregulation of oxidative stress ,a pathogenic factor of type 2 diabetes .Whether serum Mn and oxidative stress contribute to the association between dietary Mn and type 2 diabetes has not been reported. No data in humans have been reported to explain the possible mechanism of their association. It is important to clarify these connections to understand the effect of dietary Mn on type 2 diabetes. In epidemiological research, mediation analysis has been paid much attention

recently when assessing whether and how the effect of an exposure on an outcome could be explained by an intermediate variable .It can be employed to explore the mediators in the association between dietary Mn and type 2 diabetes [99].

In 2018 Longman Li and Xiaobo Yang found Mn is both a toxic and an essential trace element involved in human health and development. In the current literature, research supports a view that a U-shaped association exists between Mn, either deficiency in dietary Mn or excessive Mn exposure, and increased ROS generation as well as oxidative stress, which might affect the occurrence of metabolic diseases further, although it remains inadequate in molecular and epidemiological data on disease patients, especially among Mn workers [100].

Aims of the study:

1. Estimation levels of serum Zn ,P , Ch and Mn in diabetes mellitus type 2 patients and compare them with control group.

2. Evaluation the impact of these levels on the severity of the disease, and determine the correlation between them.

3. Comparison of two different methods in determination of serum trace elements.

Chapter Two

Materials

and

Methods

2. Materials and Methods

2.1.Instruments and Equipment

The instruments and equipment used in this study were shown in Table 2-1.

Table 2-1 : Instruments and equipment used in the study.

No	Instruments and equipment	Company / Country
1	Electrothermal atomizers (ETAs)	Japan
2	Spectrophotometer	Cecil 7200
3	Fully Auto Biochemistry Analyzer	Selectra ProXL/Netherlands
4	Eppendorf tube (1.5ml)	China
5	Centrifuge	Japan
6	Freezer	Japan
7	Gel tube	Al-Rawan/ Iraq
8	Micropipettes(100-1000µl)	Germany
9	Pipettes tips (0.02-1ml)	China
10	Distillator	GFL/Germany
11	Refrigerator	Ashtar / Iraq
12	Disposable syringe (5 ml)	China

2.2.Chemicals

All the chemicals and the standard kits utilized in the present study were shown in Table 2-2.

Table 2-2 : Chemical used in the study.

1	Glucose Kit	Linear/Spain
2	Urea Kit	spinreact/Spain
3	Creatinine kit	Linear/Spain
4	Zinc Kit	Biosam/UAE
5	Phosphorus Kit	Biosam/UAE
6	Standard Solution of Zinc(1000ppm)	Merck/Germany
7	Standard Solution of Chromium(1000)	Merck/Germany
8	Standard Solution of Manganese	Merck/Germany

2.3.Study design

This study was conducted on 180 subjects attending Al- Suwayrah hospital between October to December 2021. Subjects had age range within 35-69 years. This study is case-control study with sample size (180) include (90)T2DM patients(56 female and 34 male)with normal renal function and (90)apparently healthy control group, sample size was calculated by sample size equation in the community health department in the college. A questionnaire was designed to obtain the information from patients and control group ; which included name, age, gender, history and the presence of chronic diseases, as shown in Table 2-3.

Table 2-3 : Questionnaire used in this study.

Name:	Sample No:
Age:	
Gender:	
Date of onset of diabetes:	
Family history:	
Present history (any medical problem or medication):	
Type of treatment:	Oral: Insulin:
Smoking:	
Weight:	Height:
BMI:	
Other chronic disease:	

2.4.Exclusion Criteria

1.Type 1 diabetic patients.

2. Patients with kidney diseases.

3. Patients with skin diseases like vitiligo.

4. Pregnant women.

5. Patients with heart diseases like CVD.

2.5.Blood samples collection and storge

Three to five milliliters of blood were obtained from diabetic patients and control, then collected In tube without anticoagulants and were left for 15 minutes at room temperature to clot . After that , the blood samples were centrifuged for 10 minutes. Then the sera were aspirated and stored at (-20 ^{o}C) until time of use.

2.6.Calculation of BMI

Body mass index is calculated by dividing weight(Kg) by height square (m^2),(BMI=weight Kg/(height)^2m^2[101].

- BMI < 18kg/m^2 is underweight.
- BMI 19-24.9 kg/m^2 is normal.
- BMI 25 - 29.9kg/m^2 is overweight.
- BMI 30 - 34.9 kg/m^2 is class I obesity
- BMI 35 - 39.9 kg/m^2 is class II obesity.
- BMI 40 kg/m^2 and above is Class III obesity.

2.7. Methods

2.7.1.Spectrophotometric Determination of serum Glucose Concentration

2.7.1.1.Principle

In the Trinder reaction1,2, the glucose is oxidized to D-gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of phenol and 4-aminoantipyrine (4-AA) is oxidized by hydrogen peroxide, to form a red quinoneimine dye proportional to the concentration of glucose in the sample.

β-D-Glucose + H_2O + O_2 → D-Gluconate + H_2O_2

4-AA + Phenol→ Quinonimine + H_2O

2.7.1.2.Reagent Composition

R1

Monoreagent: Phosphate buffer 100 mmol/L pH 7.5,glucose oxidase (10 KU/L), peroxidase (2 KU/L), 4-aminoantipyrine 0.5 mmol/L, phenol 5 mmol/L.

CAL

Glucose standard: Glucose 100 mg/dL (5.55 mmol/L).Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 917b.

2.7.1.3.Reagent Preparation

The Monoreagent and the Standard are ready-to-use.

2.7.1.4.Samples

Serum or heparin plasma free of hemolysis. Glucose is stable up to 24 hours at 2-8ºC when serum or plasma is separated within 30 minutes after collection.

2.7.1.5.Materials Required

- Photometer or colorimeter capable of measuring absorbance at 500 ± 20 nm.

-Constant temperature incubator set at 37ºC.

-Pipettes to measure reagent and samples.

2.7.1.6.Procedure

1.Reagents and samples were brought to room temperature.

2.Labelled tubes were pipetted.

Table 2-4 : The Additions

TUBES	Blank	Sample	CAL.Standard
R1. Monoreagent	1.0 mL	1.0 mL	1.0 mL
Sample	----	10 µL	----
CAL.Standard	----	----	10 µL

3.The tubes were mixed and stand 10 minutes at room temperature or 5 minutes at 37ºC.

4.The absorbance (A)was read of the samples and the standard at 500 nm against the reagent blank (The color is stable for about 2 hours protected from light).

2.7.1.7.Calculation

A Sample

———— x C Standard = mg/dL glucose

A Standard

2.7.2.Spectrophotometric Determination of serum Urea Concentration

2.7.2.1.Principle

Urea in the sample is hydrolyzed enzymatically into ammonia (NH^+_4)and carbon dioxide (CO_2). Ammonia ions formed reacts with α -Ketoglutarate in a reaction catalyzed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to NAD^+:

Urea + H_2O +$2H^+$ → NH^+_4 + CO_2 by urease NH^+_4

NH^+_4 + α –Ketoglutarate +NADH →H_2O+ NAD^++L-Glutamate

The decrease in concentration of NADH, is proportional to urea concentration in the sample.

2.7.2.2.Reagent Composition

Table 2-5 : Reagent Composition.

R1 Buffer	TRIS PH 7.8 α –Ketoglutarate Urease	80 mmol/L 6mmol/L 7500 U/L
R2 Enzymes	GLDH NADH	60000 U/L 0.32 mmol/L

2.7.2.3.Reagent Preparation: All the reagents are ready to use.

2.7.2.4.Samples

.Serum or heparinized plasma .

.Urine : Sample was diluted 1/50 in distilled water. The results were multiplied by 50 (DILUTION Factor). Urine samples were preserved at PH<4.

*Urea was stable at 2-8ºC for 5 days.

2.7.2.5.Additional Equipment

-MINDRAY BS-120/ BS-200E Autoanalyzer.

-General Laboratory equipment.

2.7.3.Spectrophotometric Determination of serum Creatinine Concentration

2.7.3.1.Principle

This procedure is based upon a modification of the original picrate reaction (Jaffe)1. Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid→ Red addition complex

2.7.3.2.Reagent Composition

R1: Picric acid: Picric acid 25 mmol/L

R2: Alkaline buffer: Phosphate buffer 300 mmol/L pH 12.7,SDS 2.0 g/L (w/v). **Xi R:36/37/38**

CAL: Creatinine standard: Creatinine 2 mg/dL (177 µmol/L).

Organic matrix based primary standard. Concentration value is traceable to Standard Reference Material 914a.

2.7.3.3.Reagent Preparation

Working reagent. 1 volume of R1 + 1 volume of R2. Stable for 1 week at room temperature, stored tightly closed and protected from light

2.7.3.4.Samples

Serum or heparinized plasma, and urine. Creatinine in serum or plasma is stable up to 24 hours at 2-8ºC. Freeze for longer storage. Creatinine from random samples of urine is stable for 4 days at 2-8ºC. Freeze for longer storage. The 24-hour urine samples for the clearance test

should be collected on a preservative (fluoride thymol)and immediately refrigerated.

2.7.3.5.Materials Required

-Photometer or colorimeter with a thermos tatted cell compartment, able of reading at 510 ± 10 nm.

-Constant temperature incubator set at 37ºC.

-Stopwatch, strip-chart recorder or printer.

-Cuvettes with 1-cm pathlength.

-Pipettes to measure reagent and samples.

2.7.3.6.Procedure

1. Working reagent, samples and standard were preincubated to reaction temperature (37ºC).

2. The photometer was set to 0 absorbance with distilled water.

3. A cuvette was pipetted.

Table 2-6 : The Additions**.**

Working reagent	1.0 mL
Sample or Standard	100 µL

4. Cuvette was inserted into the temperature-controlled instrument and was started stopwatch.

5. Absorbance was recorded at 510 nm after 30 seconds (A1) and after 90 seconds (A2) of the sample or standard addition.

2.7.3.7.Calculations: For Serum and plasma :

$$\frac{(A2 - A1)\ \text{Sample}}{(A2 - A1)\ \text{Standard}} \times C\ \text{Standard} = \text{mg/dL creatinine}$$

Samples with concentrations higher than 20 mg/dL should be diluted 1:4 with saline and assayed again. The results were multiplied by 4.If results were to be expressed as SI units apply: mg/dL x 88.4 = µmol/L.

$$\text{Clearance Test mL/min} = \frac{\text{mg creatinine/ dL URINE x mL 24-h}}{\text{mg creatinine/ dL SERUM x 1440 min}}$$

2.7.4.Spectrophotometric Determination of serum Phosphorus Concentration

2.7.4.1.Principle

Phosphate ions in an acidic medium react with ammonium molybdate to form a phosphomolybdate complex. This complex has an absorbance in ultraviolet range and is measured at 340 nm. Intensity of the complex formed is directly proportional to the amount of inorganic phosphorus present in the sample.

Phosphorus + Ammonium Molybdate → phosphomolybdate complex

2.7.4.2.Reagent Composition

Acid Reagent: Sulphuric acid 700mM

Molybdate Reagent: Ammonium molybdate 0.4 mM

Detergent

2.7.4.3.Reagent Preparation: Reagents are ready to use.

Working reagent: The contents of 1 bottle of Molybdate Reagent was poured into 1 bottle of Acid Reagent. This working reagent was stable for at least 6 months when stored at 2-8ºC. Upon storge the working reagent may develop a slight blue colour however this does not affect the performance of the reagent. Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of Acid Reagent and 1 part of Molybdate Reagent. Alternatively 0.8 ml of Acid Reagent and 0.2 ml of Molybdate Reagent may also be used instead of 1ml of the working reagent directly during the assay.

2.7.4.4.Samples

Serum , Heparinized /EDTA Plasma or urine . Acidify the urine with a few drops of conc. Hydrochloric acid and dilute 1+19 before the assay,(Results ×20). Inorganic phosphorus is reported to be stable in serum for 7 days at 2-8ºC.

2.7.4.5.Materials Required

Photometer analyzer with standard thermostatic cuvette holder , micropipette and appropriate laboratory equipment.

2.7.4.6.Procedure

Table 2-7 : The Additions.

Addition Sequence	B (ml)	S (ml)	T (ml)
Working Reagent	1.0	1.0	1.0
Distilled Water	0.01	---	---
Phosphorus Standard	---	0.01	---
Sample	---	---	0.01

The Additions were mixed well and incubated at (25ºC) for 5 mins. The absorbance was measured of the Standard (Abs.S), and Test Sample (Abs.T)against the Blank ,with 60 mins.

2.7.4.7.Calculations

$$\text{Phosphorus in mg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 5\text{mg/dl concentration of S.}$$

2.7.5.Spectrophotometric Determination of serum Zinc Concentration

2.7.5.1.Principle

Zinc in an alkaline medium reacts with Nitro-PAPS to form a purple colored complex. Intensity of the complex formed is directly proportional to the amount of zinc present in the sample.

Zinc + Nitro-PAPS → Purple Colored Complex in Alkaline Medium

2.7.5.2.Reagent Composition

- Borate Buffer 290mM .

-PH 8.2 .

-Salicilaldoxime 10 mM.

-Dimethylglyoxime 1.0 mM.

-NITRO-PAPS 0.08 mM.

-Surfactants and Preservatives.

2.7.5.3.Reagent Preparation: Reagents are ready to use.

Working reagent: The contents of 1 bottle of Colour Reagent was poured into 1 bottle of Buffer Reagent. This working reagent was stable for at least 2 weeks when stored at 2-8ºC. Alternatively for flexibility as much of working reagent may be made as and when desired by mixing together 4 parts of Buffer Reagent and 1 part of Colour Reagent. Alternatively 0.8 ml of Buffer Reagent and 0.2 ml of Colour Reagent may also be used instead of 1ml of the working reagent directly during the assay.

2.7.5.4.Samples

Serum (Free from hemolysis)or urine . Zinc is reported to be stable in serum for 7 days at 2-8ºC.

2.7.5.5.Materials Required

Photometer analyzer with standard thermostatic cuvette holder , micropipette and appropriate laboratory equipment.

2.7.5.6.Procedure

Table 2-8: The Additions.

Addition Sequence	B (ml)	S (ml)	T (ml)
Working Reagent	1.0	1.0	1.0
Distilled Water	0.05	---	---
Zinc Standard	---	0.05	---
Sample	---	---	0.05

The Additions were mixed well and incubated at (25ºC) for 5 mins. The absorbance was measured of the Standard (Abs.S), and Test Sample (Abs.T)against the Blank ,with 20 mins.

2.7.5.7.Calculations

$$\text{Zinc in } \mu g/dl = \frac{Abs.T}{Abs.S} \times 200 mg/dl \text{ concentration of S.}$$

2.7.6.Quantitative measurement of elements (Ch ,Mn ,Zn) in serum

2.7.6.1.Principle:[102-104]

Flameless Atomic absorption spectrophotometer method by the technique of Graphite Furnace (GFAAS) was used to determine the trace element(Ch,Mn,Zn).SHIMADZU AA7000 Atomic Absorption Spectrophotometer was used for determination of these elements. GFAAS is one of the most important of the five techniques of the atomic absorption spectrometry in which has the higher sensitivity which can be reach to the low detection limits (in ppb units).

This technique is also called Electro thermal Atomic Absorption Spectrometry (ETAAS) which is a type of spectrometry that uses a graphite furnace tube to vaporize the sample in three stages, drying, ashing, and atomizing.

The fact of this technique is based on that free atoms of element absorb light produced from the specific cathode lamp at specific wavelengths characteristic of the interest element. Within certain limits, the amount of light absorbed reflect the concentration of analyst present and can be linearly correlated to this concentration. Most elements can produce free atoms from samples by the application of high temperatures.

In GFAAS, very small amount of samples (10µL-20µL) is injected in small graphite or paralytic carbon coated graphite tube, which can then be heated by a wide range of temperature to vaporize and atomize the analyst. The atoms absorb the electromagnetic radiation in the ultraviolet or visible region resulting in transitions of electrons to higher electronic energy levels to the excited state and then back to the ground state by emitting it's specific characteristic light which can be measured to determine the samples concentrations. The temperature of the Graphite

tube increases over a matter of seconds and can reach up to 3000ºC depending on the element being analyzed.

2.7.6.2.Preparation of Standard Solutions:

Original standard solutions were (1000µg/ml in 2% HNO3) for interested elements. Four standard solutions were prepared by dilution from original standard stock solution using general dilution law ($C_1 V_1 = C_2 V_2$). It must prepare a series of concentrations from the highest one reaching the values required for calibration curve performs. Series of concentrations which prepared were as follows:-

- (1000µg/ml → 100µg/ml → 10µg/ml → 1µg/ml).

The value 1µg/ml (1 ppm) is equal to 1000ng/ml (1000 ppb), so complete preparation from this solution reaching the required concentration of the standard calibration curve as follow:-

- (1000ng/ml → 100ng/ml → prepare the needed concentration of any element).

2.7.6.3.Sample Preparation:

Samples were digested by transferring (20µl) of serum into Eppendorf tube , then (40µl)of 10% nitric acid was added and mixed well for 10 minutes, finally complete dilution volume to (100µl) with deionized water. Solutions was filtered and then appropriate solution volume of (20µl) was injected into the graphite furnace tube for reading.

2.7.6.4.Determination of serum chromium:

Four standard solutions of the element were prepared as previously mentioned. The four standards were (5, 10, 20, 30 ng/ml) for calibration curve as shown in figure 2-1. The concentrations of chromium in samples

were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve.

2.7.6.5.Conditions for Ch Determination: Listed in Table 2-9 .

Table 2-9 : Ideal Conditions for Ch Determination**.**

Variable	**Ideal condition**
Lamp current	10 mA
Wavelength	357.9 nm
Slit width	0.5 nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

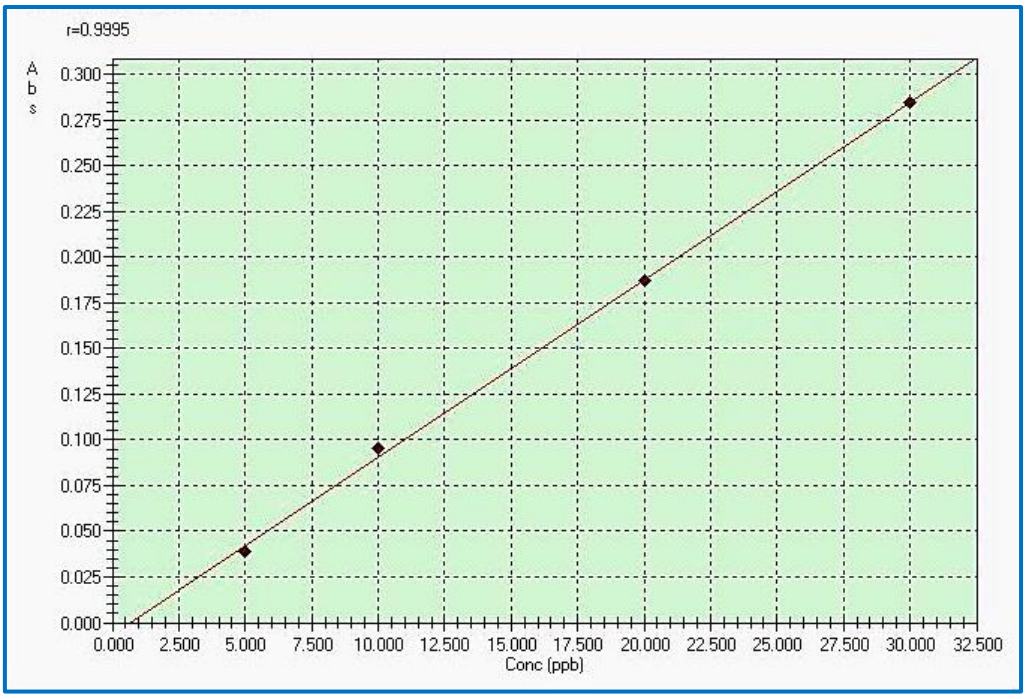

Figure 2-1: Standard Curve for de Ch determination.

2.7.6.6.Determination of serum Mn:

Five standard solutions of the element were prepared as previously mentioned. These standards were (2.5, 5, 10, 15, 20, ng/ml) for calibration curve as shown in figure 2-2. The concentrations of manganese in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve.

2.7.6.7.Conditions for Mn Determination: Listed in Table 2-10.

Table 2-10 : Ideal Conditions for Mn Determination.

Variable	**Ideal condition**
Lamp current	10 mA
Wavelength	279.5 nm
Slit width	0.2 nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

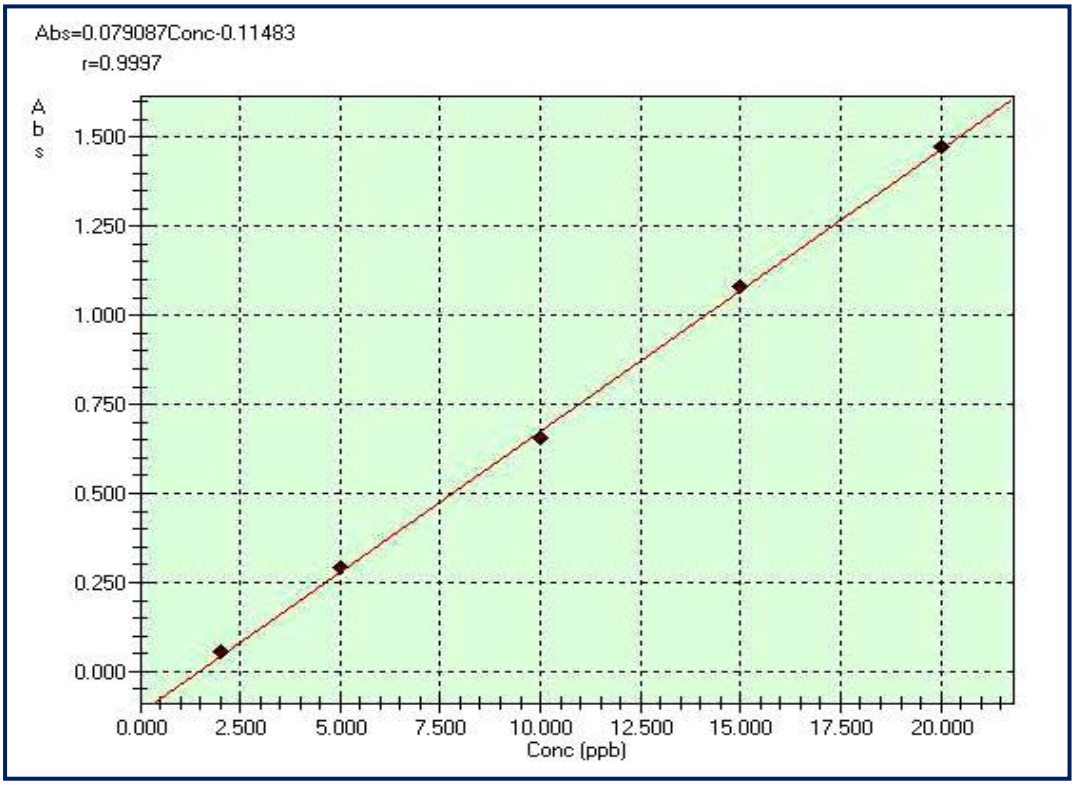

Figure 2-2: Standard Curve for Mn determination.

2.7.6.8.Determination of serum Zn:

Five standard solutions of the element were prepared as previously mentioned. These standards were (2, 4, 6, 8, 10 ng/ml) for calibration curve as shown in figure 2-3. The concentrations of zinc in samples were measured directly and continuously beyond measuring of standard solutions depending on the calibration curve.

2.7.6.9.Conditions for Zn Determination: Listed in Table 2-11.

Table 2-11: Ideal Conditions for Zn Determination**.**

Variable	**Ideal condition**
Lamp current	8 mA
Wavelength	213.9 nm
Slit width	0.5 nm
Lighting mode	BGC-D2
Sample Size	20 µl
Replicates	3

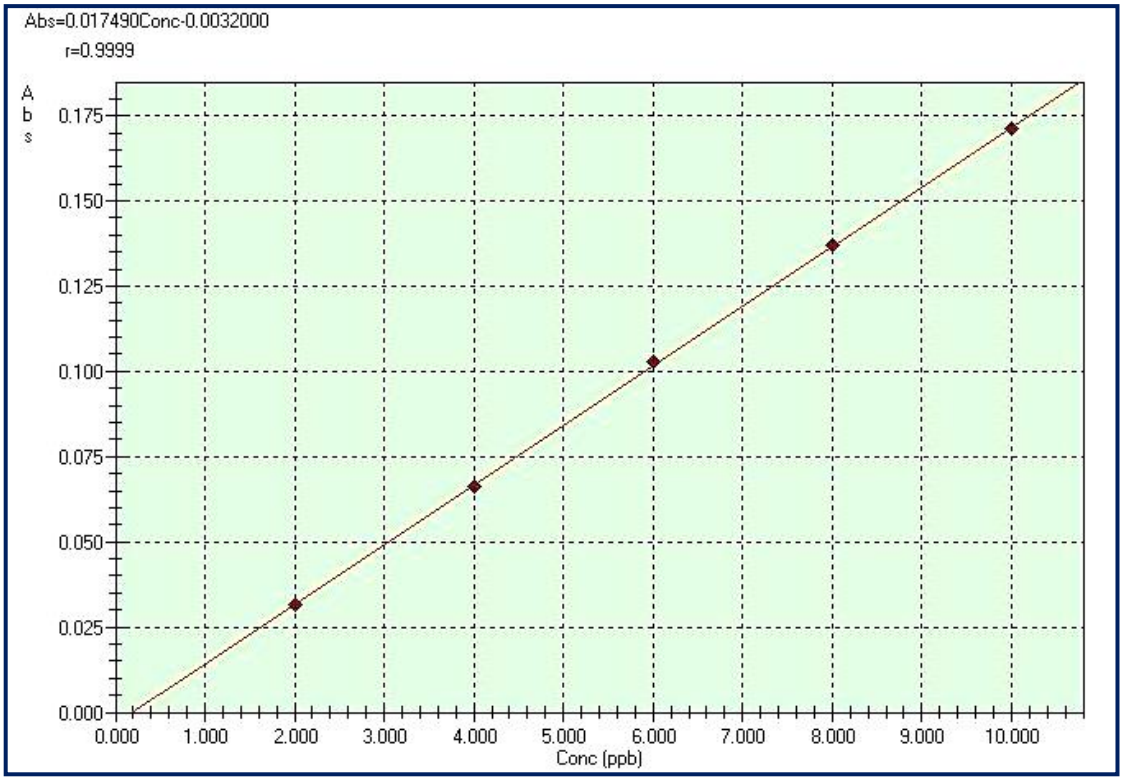

Figure 2-3 : Standard Curve for Zn determination.

2.8. Statistical Analysis

Statistical analysis was carried out using SPSS version 25. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. Paired t-test was used to compare means for two paired readings. A p-value of ≤ 0.05 was considered as significant [105].

Chapter Three

Results

and

Discussion

3.Results and Discussion

3.1. Age, Gender and BMI in Diabetes Mellitus type 2

This study enrolled, ninety patients suffered from diabetes Mellitus type 2(case group), 34 (37.8%) were males and 56 (62.2%) were females. The control group was ninety adults,34 were males and 56 were females with mean age and body mass index ,as shown in Table 3-1 or figure 3-1 shows distribution of diabetic patients according to study variables including (age, gender and body mass index). Mean age of patients was (54.51 ± 6.69) years, minimum age was 40 years and maximum age was 69 years. Majority of patients (62.2%) were female and majority of patients (73.3%) were obese.

Table 3-1: The Distribution of patients according to study variables (N=90).

Study variables	(Means ± SD)	
Age (years)	(54.51 ± 6.69)	(40.0 - 69.0)%
Gender		
Male	34	37.8%
Female	56	62.2%
Total	90	100.0%
BMI (kg/m^2)		
Normal (18.5-24.9)	0	0.0%
Overweight (25-29.9)	24	26.7%
Obese (≥ 30)	66	73.3%
Total	90	100.0%

Mean body mass index was (32.92 ± 4.26)

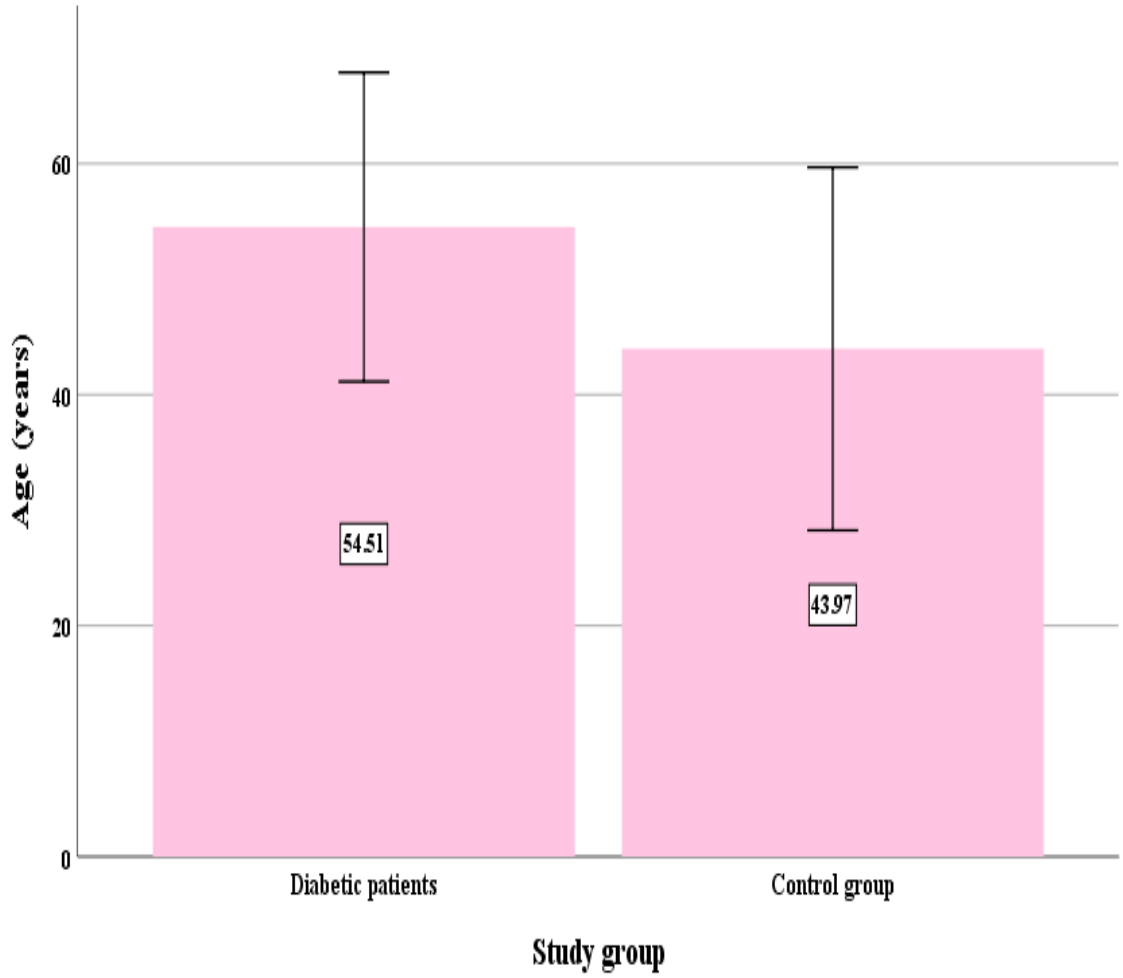

Figure 3-1 : The mean differences of age according to study group.

The mean differences of age and body mass index according to study group including (diabetic patients and control group).There were significant differences between means of age and body mass index according to study group, as shown in Table 3-2 or figure 3-2 and as the following studies show, in the United States, the average age of diagnosis of type 2 diabetes mellitus has reduced from 52 years in the years 1988 to 1994 to 46 years in the years 1999 to 2000. There are a number of probable causes for the ten-year drop. This shift could indicate an earlier start of type 2 diabetes, earlier identification, or a combination of the two.

It is uncertain whether the decrease in age at diagnosis was attributable to a reduction in the actual age of onset among American adults or to earlier detection of type 2 diabetes by physicians, and this cannot be determined from the data. Decreasing the age at diagnosis could also be the result of increasing public knowledge, which could lead to earlier physician consultations concerning recognized diabetic symptoms, which could represent better population education about diabetes risks [106].

They discovered that being overweight or obese is a substantial contributor to type 2 diabetes and its consequences in both men and women. Both men and women in the overweight range($25 \leq BMI \leq 29.99$) had a 30% and 10% higher chance of acquiring diabetes, respectively. At($30 \leq BMI \leq 39.99$), both genders were 100 percent more likely to develop diabetes than those with a normal BMI. These findings point to a larger link between BMI and the onset of diabetes than has previously been shown in similar investigations [107].

Table 3-2: The mean differences of age and body mass index according to study group.

Study variables	**Study group**	**N**	**Mean ± SD**	**t-test**	**P-value**
Age (years)	Diabetic patients	90	54.51 ± 6.69	**9.694**	**<0.001***
	Control group	90	43.97 ± 7.85		
BMI (Kg/m^2)	Diabetic patients	90	32.92 ± 4.26	**7.366**	**<0.001***
	Control group	90	29.05 ± 2.56		

*P value ≤ 0.05 was significant

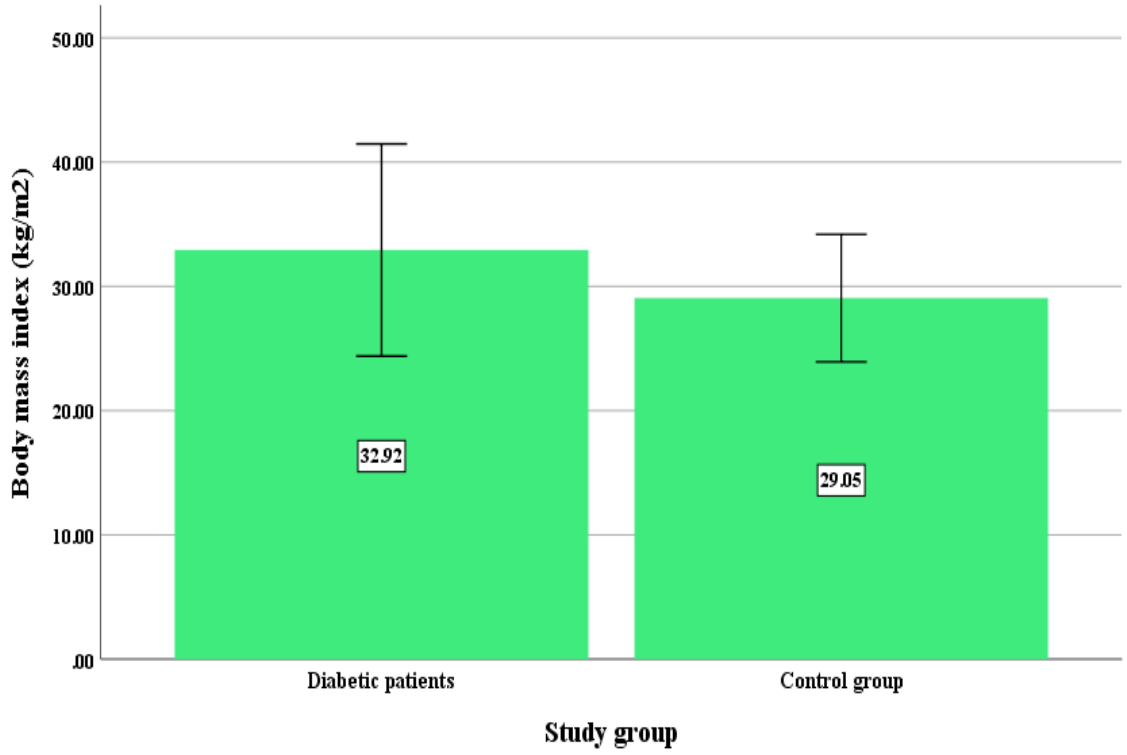

Figure 3-2: The mean differences of BMI according to study group

3.2.Fasting plasma Glucose, Urea and Creatinine in Diabetes Mellitus type2

In this study ,The mean differences of blood urea , serum creatinine and fasting plasma glucose according to study group including (diabetic patients and control group).There were significant differences between means of blood urea , serum creatinine and FPG according to study group, as shown in Table 3-3 or figures 3-3, 3-4, 3-5.

Impairment of urea and creatinine level due to increased blood glucose level indicates reduction in kidney function in diabetic patients. Diabetic kidney disease (DKD) develops in about 40% of patients who are diabetic and is the leading cause of chronic kidney disease (CKD) worldwide. Metabolic changes caused by diabetes leads to glomerular hypertrophy, glomerulosclerosis, tubulointerstitial inflammation and

fibrosis. Due to this there is large residual risk of DKD. Assessment of serum urea and creatinine impairment with associated factors is very crucial for early diagnosis and prevention of complication of diabetes mellitus [108].

Table 3-3: The mean differences of blood urea, serum creatinine and FPG according to study group**.**

Study variables	Study group	N	Mean ± SD	t-test	P-value
Blood urea/ sp (mg/dl)	Diabetic patients	90	29.36 ± 5.06	**2.096**	**0.038***
	Control group	90	27.76 ± 5.18		
Serum creatinine / sp (mg/dl)	Diabetic patients	90	0.80 ± 0.17	**2.755**	**0.006***
	Control group	90	0.73 ± 0.16		
FPG/ sp (mg/dl)	Diabetic patients	90	214.89 ± 78.33	**15.391**	**<0.001***
	Control group	90	87.31 ± 6.87		

*P value ≤ 0.05 was significant

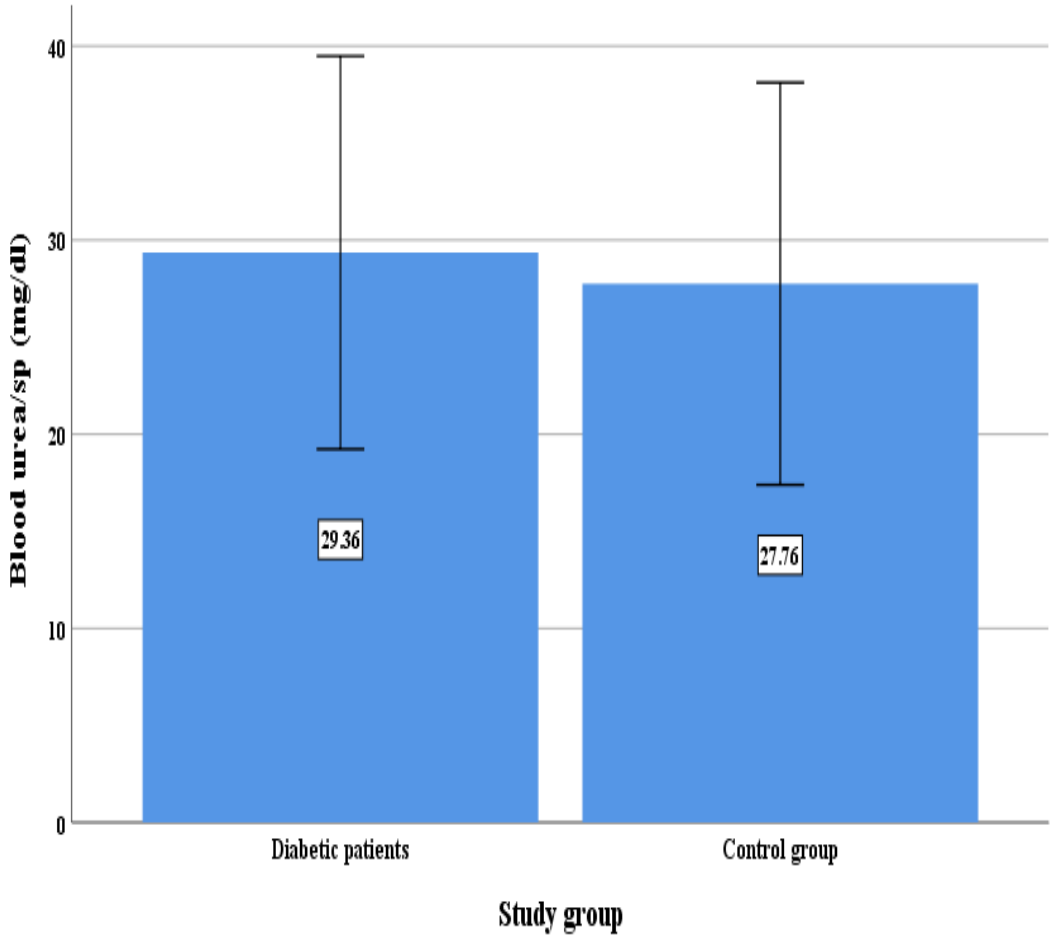

Figure 3-3: The mean differences of blood urea / sp according to study group.

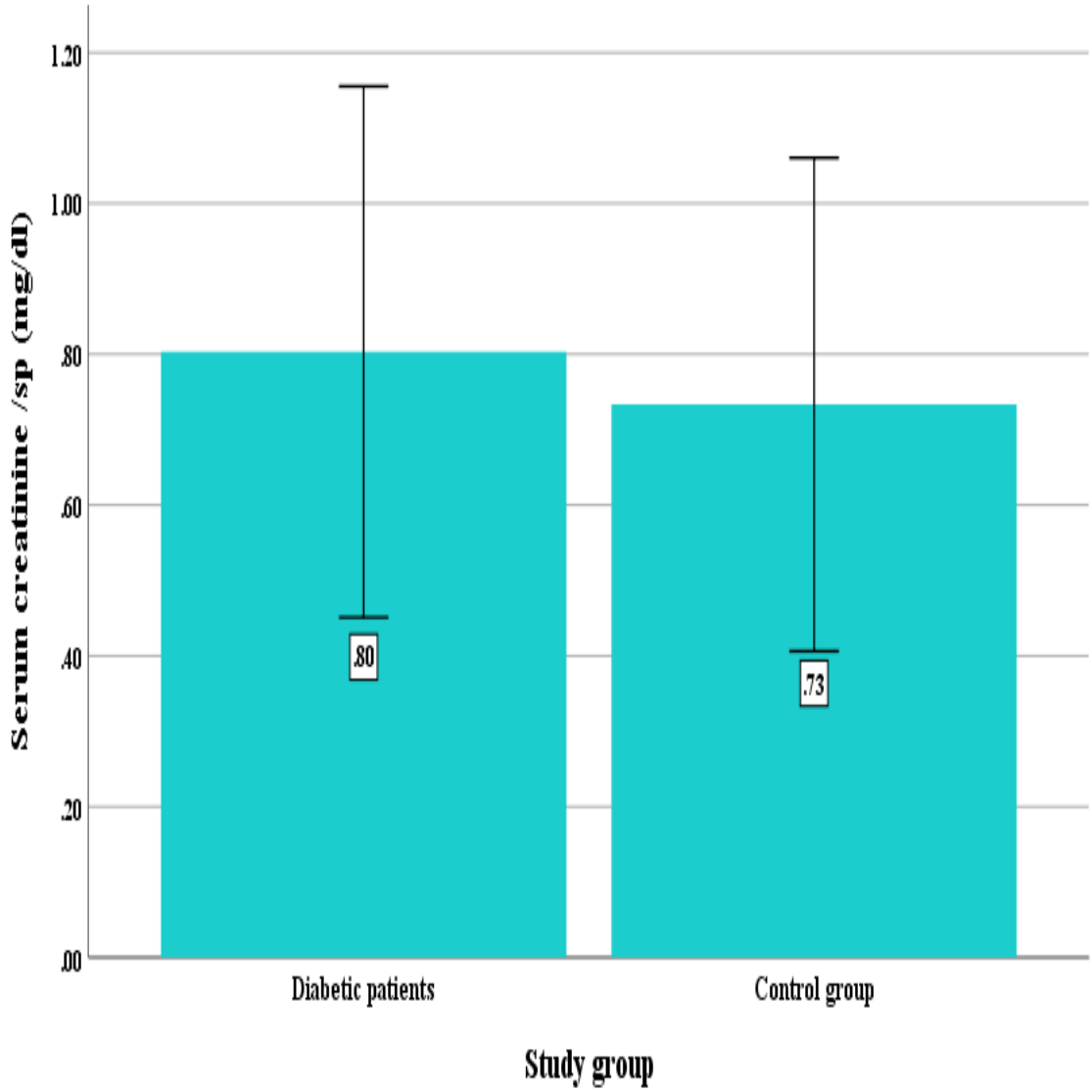

Figure 3-4: The mean differences of serum creatinine / sp according to study group.

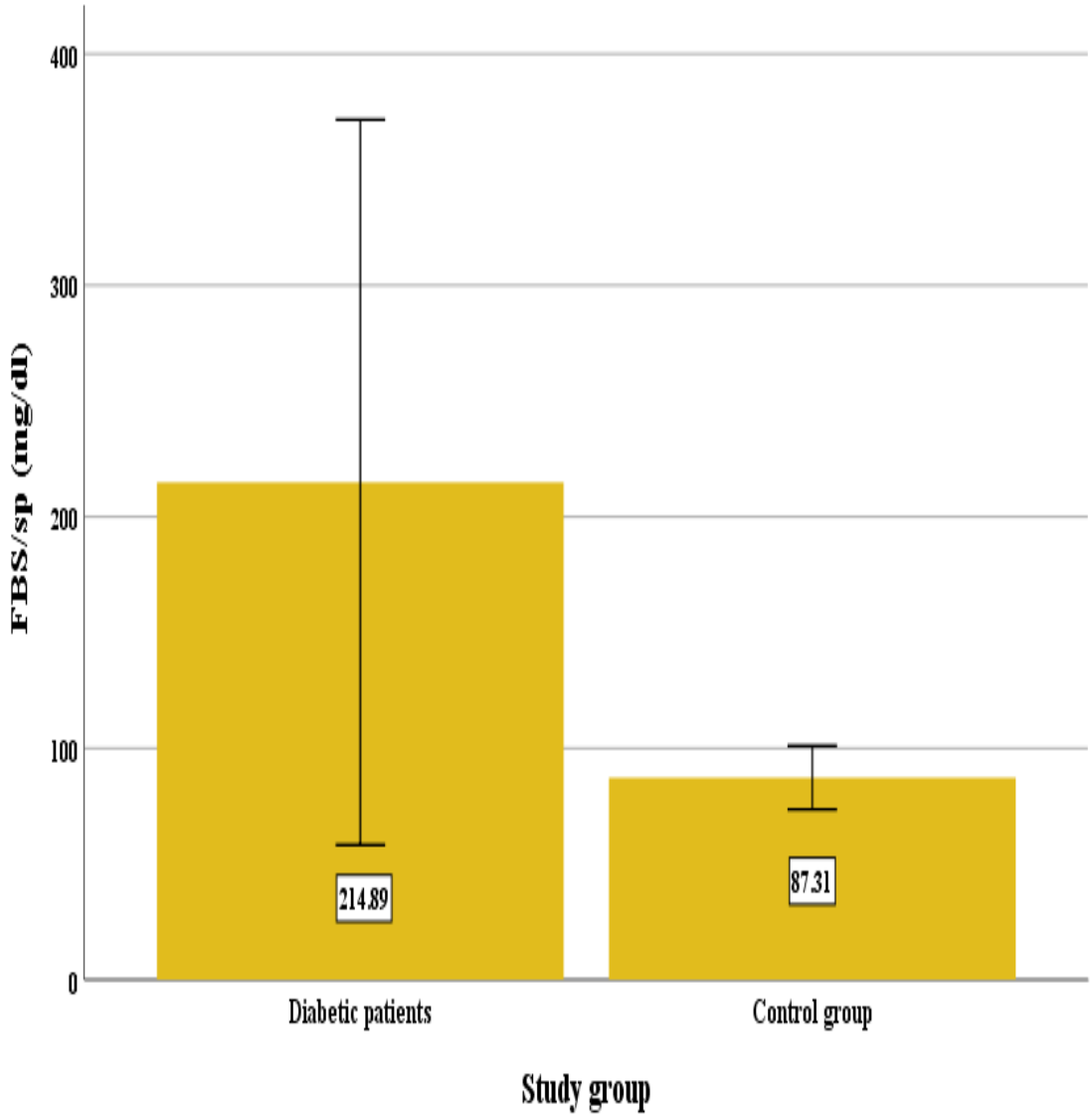

Figure 3-5: The mean differences of FPG / sp according to study group.

3.3.Zinc in Diabetes Mellitus type2

In this study, serum zinc measured by two methods spectrophotometric and atomic absorption. Distinguish between two methods there were significant differences between means of zinc according to study group P-value <0.001 (P value ≤ 0.05 was significant) ,as shown in Tables 3-4, 3-5 or figures 3-6, 3-7.

In type 2 diabetes individuals, Saharia *etal.*[109], Basaki *et al.*[110]and Jansen *etal.*[111]discovered lower zinc plasma concentrations. These findings are linked to a significant quantity of mineral loss in the urine. In these patients, glycemic management influences this loss, which is not balanced by an increase in its uptake by intestinal cells or a reduction in intestinal excretion. Hyperglycemia, according to Jayawardena, interferes with active zinc transport into renal tubular cells, resulting in hyperzincuria [112]. The results of this study approve the previous observations, zinc concentrations in diabetic type 2 individuals' blood serum are low [113].

Table 3-4: The mean differences of zinc (spectrophotometric) according to study group**.**

Study variables	Study group	N	Mean ± SD	t-test	P-value
Zinc / sp (µg/dl)	Diabetic patients	90	105.20 ± 5.82	**-5.313**	**<0.001***
	Control group	90	110.30 ± 6.99		

*P value ≤ 0.05 was significant

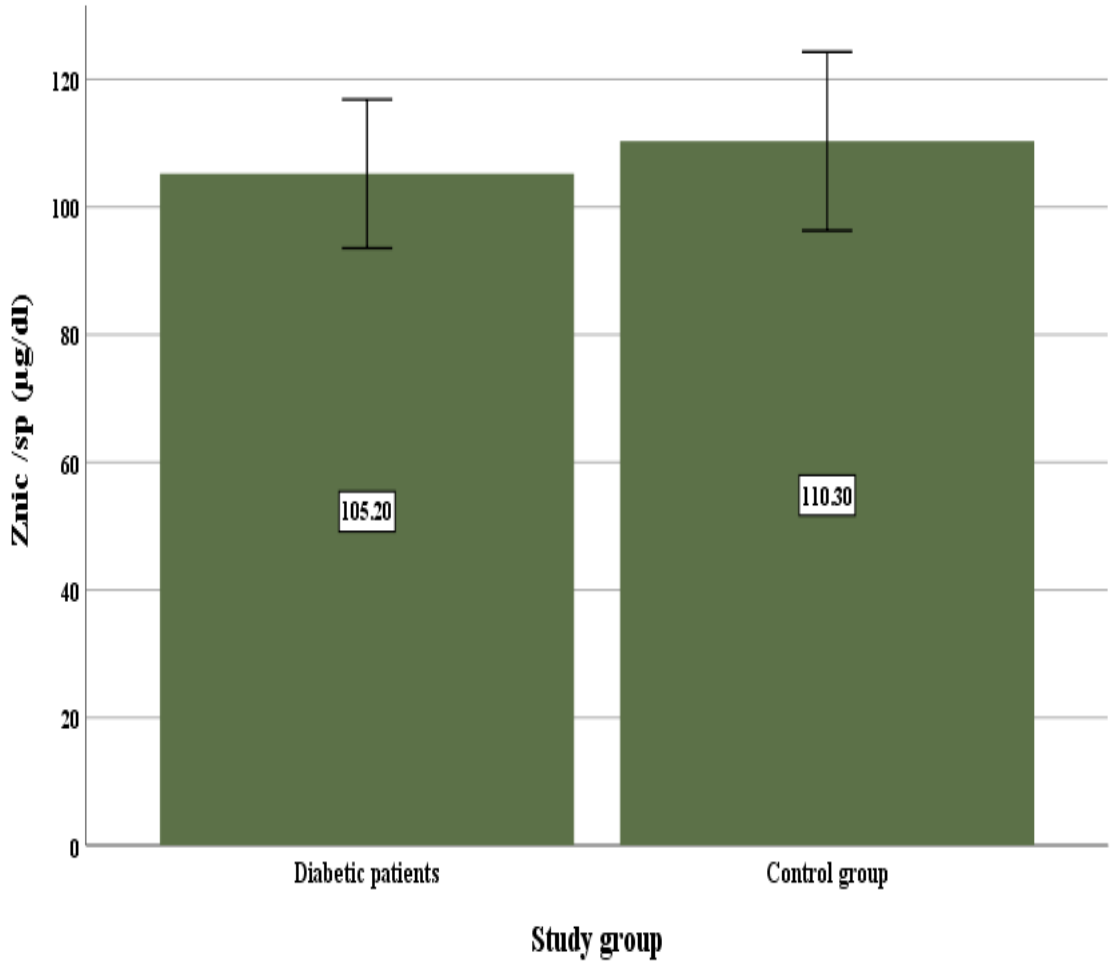

Figure 3-6: The mean differences of Zn / sp according to study group.

Table 3-5 : The mean differences of zinc (atomic absorption) according to study group.

Study variables	Study group	N	Mean ± SD	t-test	P-value
Zinc / sp (µg/dl)	Diabetic patients	90	53.79 ± 5.48	**-40.182**	**<0.001***
	Control group	90	80.64 ± 3.17		

*P value ≤ 0.05 was significant

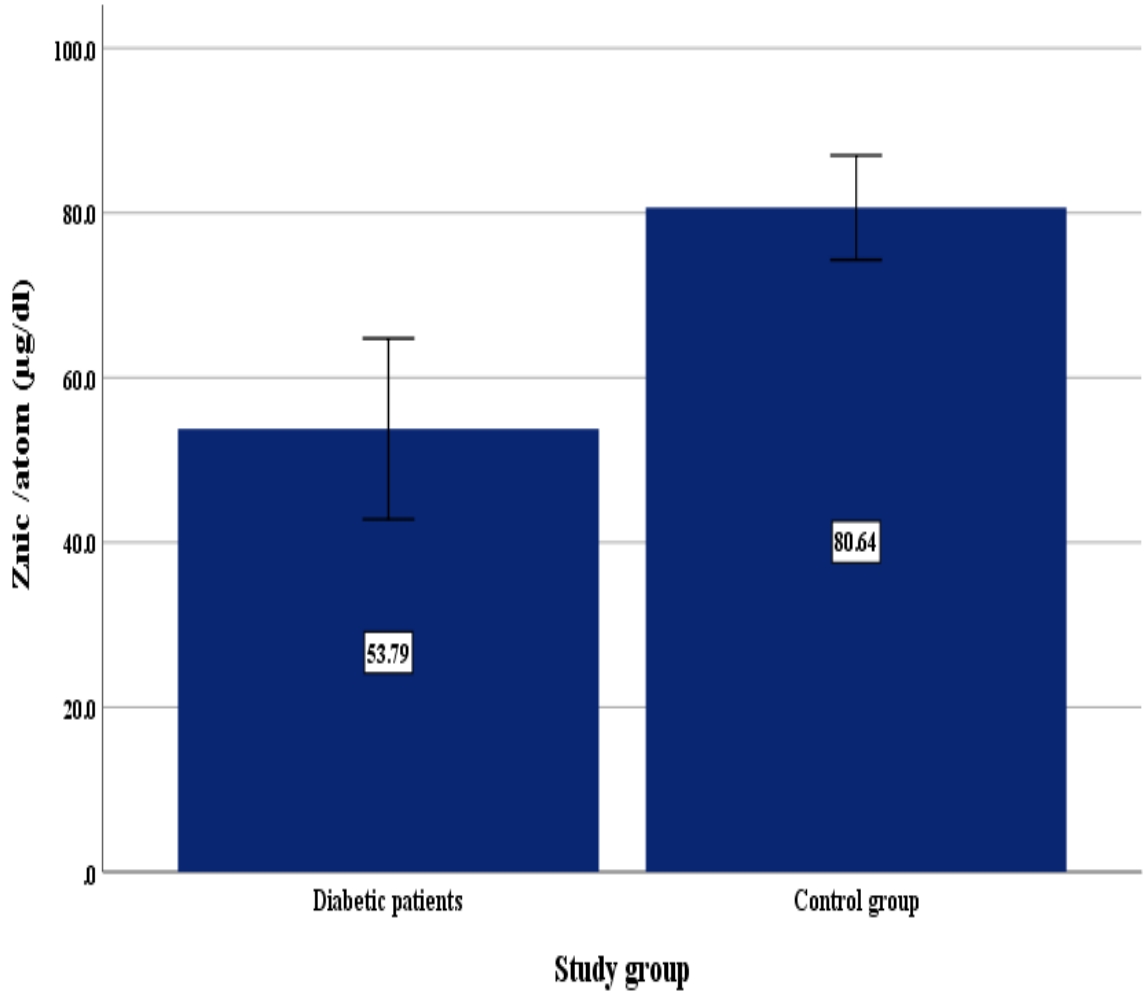

Figure 3-7: The mean differences of Zn / atom according to study group.

In this study,the mean differences of zinc (µg/dl) measured by two technique include (Spectrophotometric and Atomic absorption) among diabetic patients. There were significant differences between means of zinc between these two technique among diabetic patients, as shown in Table 3-6 or figure 3-8.

Table 3-6: The mean differences of zinc according to technique of measurement among diabetic patients (N=90).

Study variables	Study group	N	Mean ± SD	Paired t-test	P-value
Zinc (µg/dl)	Spectrophotometric	90	105.20 ± 5.82	**59.66**	**<0.001***
	Atomic absorption	90	53.79 ± 5.48		

*P value ≤ 0.05 was significant

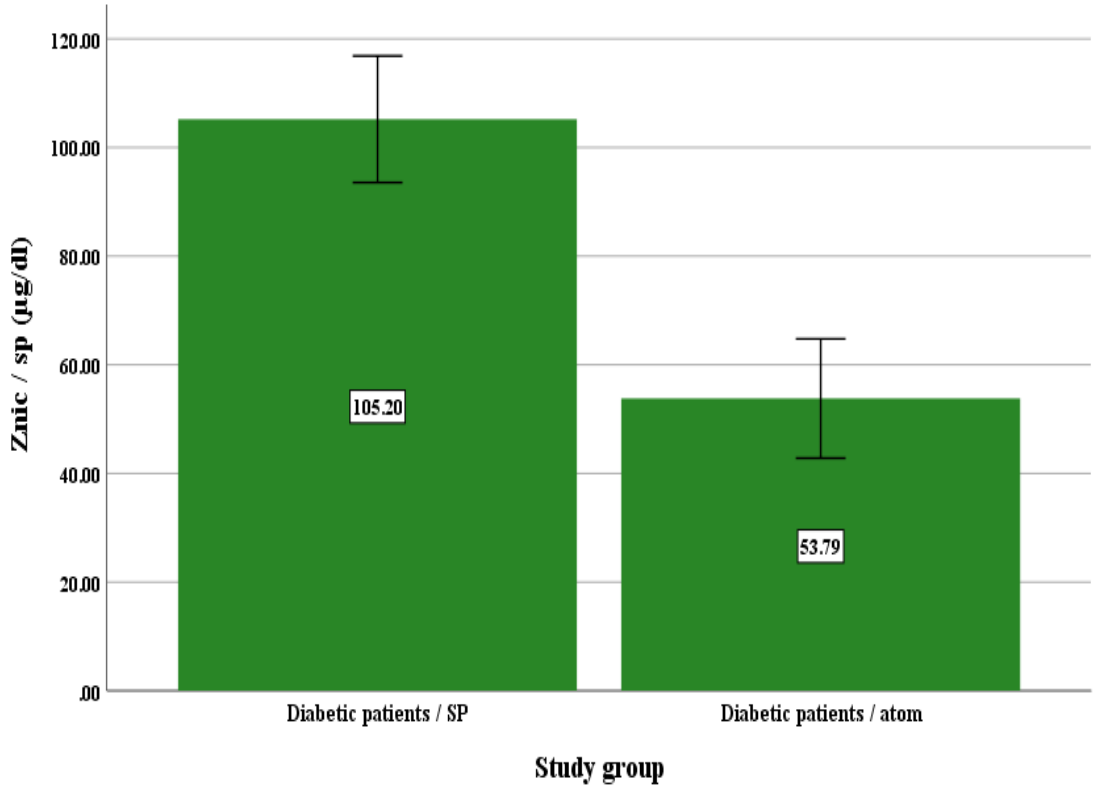

Figure 3-8: The mean differences of Zn according to technique of measurement among diabetic patients.

In this study, The mean differences of zinc (µg/dl) measured by two technique include (spectrophotometric and atomic absorption) among control group. There were significant differences between means of zinc between these two technique among control group, as shown in Table 3-7 or figure 3-9.

Table 3-7: The mean differences of zinc according to technique of measurement among control group (N=90).

Study variables	Study group	N	Mean ± SD	Paired t-test	P-value
Zinc (µg/dl)	Spectrophotometric	90	110.30 ± 6.99	**37.501**	**<0.001***
	Atomic absorption	90	80.64 ± 3.17		

*P value ≤ 0.05 was significant

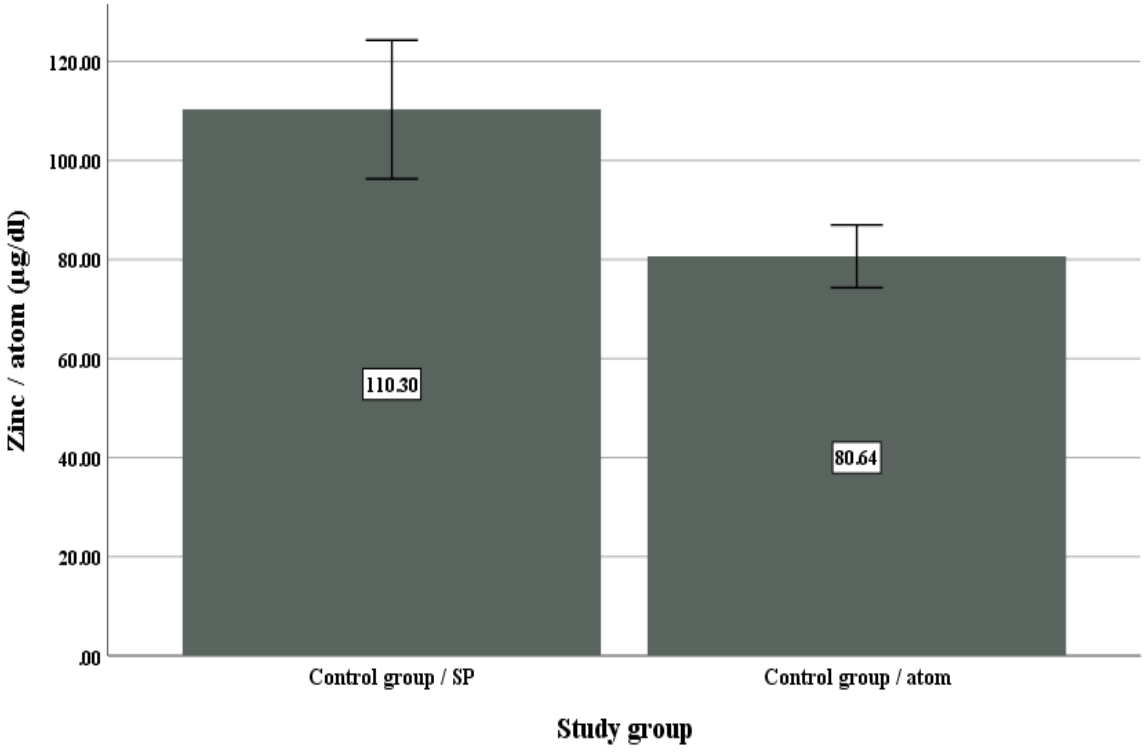

Figure 3-9: The mean differences of Zn according to technique of measurement among control group .

The ROC curve for the sensitivity and specificity of zinc / Sp (µg/dl) in diabetes mellitus, (Cut-off point was ≤ 109.50 (µg/dl)), AUC=0.71, P <0.001*, 95% CI (0.634-0.787), the sensitivity was 80.0%, the specificity was 61.1%, positive predictive value was 67.28%, negative predictive value was 75.34% and overall accuracy was 70.55%, as shown in figure 3-10.

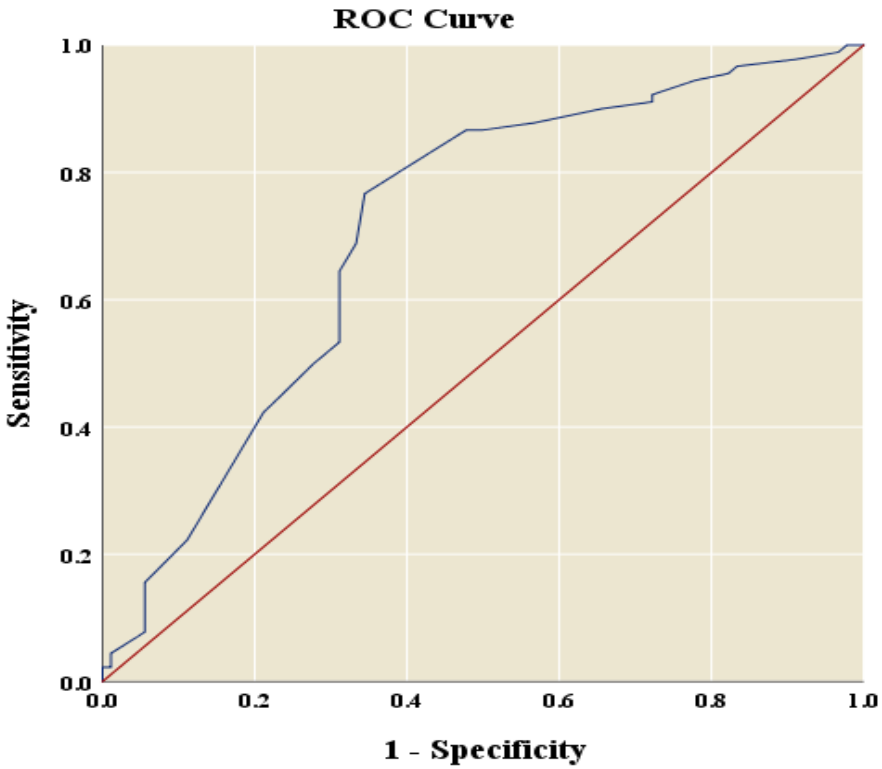

Figure 3-10 : ROC curve for the sensitivity and specificity of Zn / sp(µg/dl) in diabetes mellitus.

The ROC curve for the sensitivity and specificity of zinc / atom (µg/dl) in diabetes mellitus, (Cut-off point was ≤ 75.40 (µg/dl)), AUC=1.00, P <0.001*, 95% CI (1.000-1.000), the sensitivity was 100.0%, the specificity was 100.0%, positive predictive value was 100.0%, negative predictive value was 100.0% and overall accuracy was 100.0%, as shown in figure 3-11. AAS is the best technique for the determination of Zn in human blood serum, it is considered by many of benefits such as, simple, rapid, accurate, precise and sensitive, It is highly sensitive, with low limit of detection and less sample volume [114].

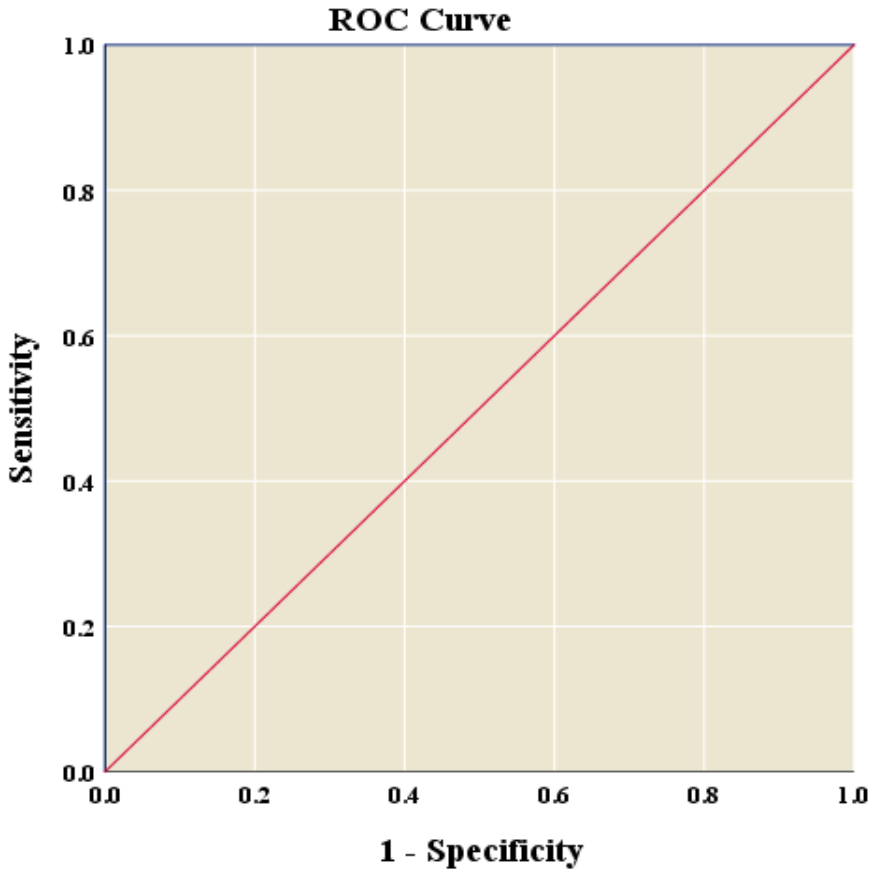

Figure 3-11: ROC curve for the sensitivity and specificity of Zn / atom (µg/dl) in diabetes mellitus.

3.4.Manganese in Diabetes Mellitus type 2

In this study, the mean differences of manganese (atomic absorption) according to study group including (diabetic patients and control group).There were significant differences between means of manganese (Atomic absorption) according to study group as shown in Table 3-8 or figure 3-12. Low level of serum Mn in diabetic group in this study. Diabetes was more common in people who had low blood manganese levels, suggesting that manganese may have a role in glucose regulation [115-117].

Table 3-8: The mean differences of manganese (atomic absorption) according to study group**.**

Study variables	Study group	N	Mean ± SD	t-test	P-value
Manganese / atom (µg/dl)	Diabetic patients	90	0.25 ± 0.03	**-79.694**	**<0.001***
	Control group	90	0.59 ± 0.02		

*P value ≤ 0.05 was significant

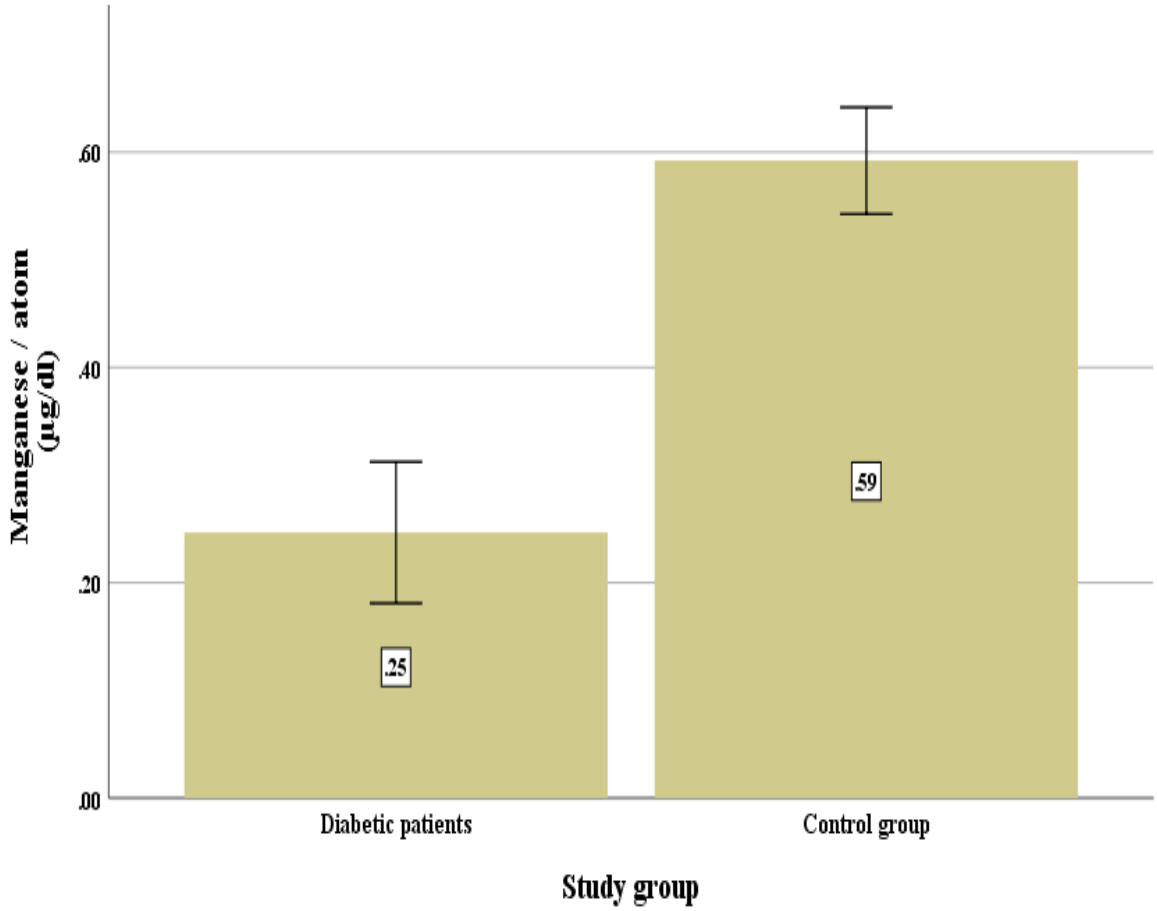

Figure 3-12 : The mean differences of Mn / atom according to study group.

3.5.Chromium in Diabetes Mellitus type 2

In this study , The mean differences of chromium (atomic absorption) according to study group including (diabetic patients and control group). There were significant differences between means of chromium (atomic absorption) according to study group as shown in Table 3-9 or figure 3-13. When compared to nondiabetic healthy control patients, Rajpathak *etal.* found that type 2 diabetics had reduced serum chromium levels. In adding to chromium, diabetics' zinc and manganese levels were shown to be lower when compared to the general population [118]. This is consistent with findings from previous research [119]. Another study found that as people got older, their serum chromium levels dropped; this matches the findings of Ding *et al.* from China, who found that senior diabetics have significantly lower serum chromium levels [120].

Table 3-9 : The mean differences of chromium (atomic absorption) according to study group.

Study variables	Study group	N	Mean ± SD	t-test	P-value
Chromium / atom (mg/dl)	Diabetic patients	90	0.20 ± 0.03	**-78.184**	**<0.001***
	Control group	90	0.82 ± 0.06		

*P value ≤ 0.05 was significant

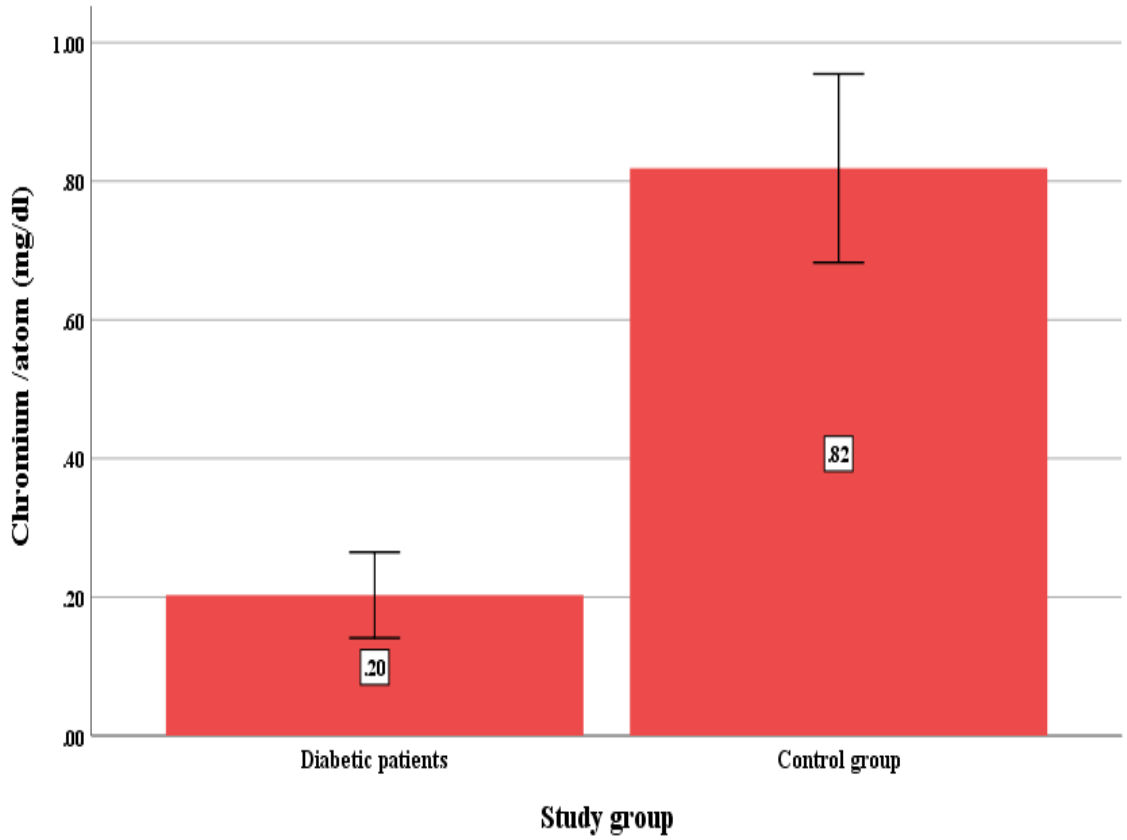

Figure 3-13 : The mean differences of Ch / atom according to study group.

3.6. Phosphorus in Diabetes Mellitus Type 2

In this study, there were no significant differences between means of serum phosphate (spectrophotometric) according to study group as shown in Table 3-4 or figure 3.6. Phosphate metabolism disturbances are rather common in diabetic patients. As early in the development of diabetic patients' plasma phosphate levels may be normal or even low, these deregulations may be difficult to distinguish.

Although individuals may proceed from initial hyperphosphatemia to hypophosphatemia to normalization of plasma phosphate, phosphate deregulations are more obvious in the context of DKA. A recent study comparing 162 type 2 DM patients to 82 hospitalized non-DM patients found that serum P levels were lower in type 2 DM patients due to metabolic disturbances [121]. In contrast, others found no evidence of lower serum P in the diabetic group compared to the non-DM group in the current study [122]. Another study found no significant differences in calcium and phosphorus levels between diabetics and healthy people [123].

Table 3-10 : The mean differences of serum phosphate (spectrophotometric) according to study group.

Study variables	Study group	N	Mean ± SD	t-test	P-value
Phosphorus / sp (mg/dl)	Diabetic patients	90	4.12 ± 0.33	-1.029	0.305
	Control group	90	4.17 ± 0.34		

*P value ≤ 0.05 was significant

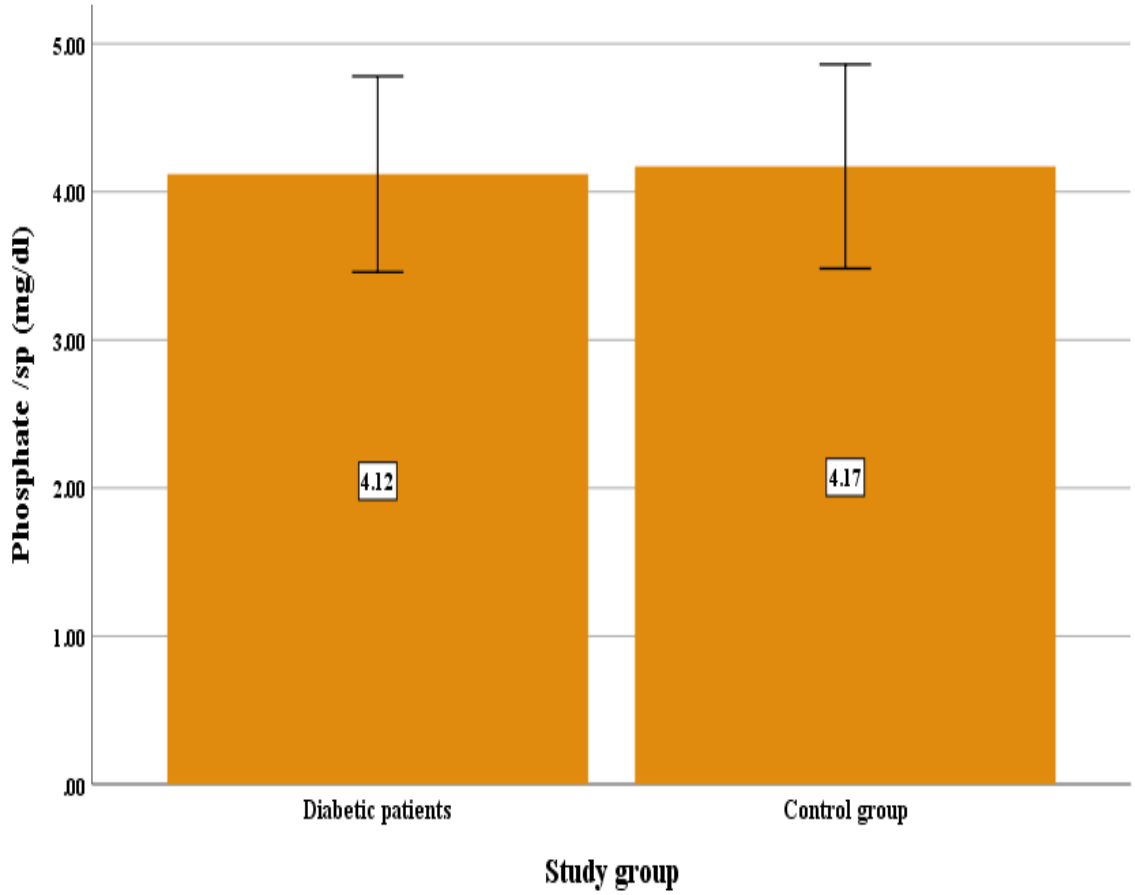

Figure 3-14: The mean differences of phosphate / sp according to study group.

In this study , determine serum phosphate by spectrophotometric method, The simple spectrophotometric method for the phosphate measurement in this work was adapted from the official phosphomolybdate method .This method is much simple, compared with the other developed method. This method provides some advantages such as, simple, easy, cheap and also carried out without any complex preparation sample procedure. Hence, this simple method is suitable for the routine determination of phosphate in common laboratories[124].

Conclusion:

1. The current study finding point to a larger link between BMI and the onset of diabetes.

2. Scientific evidences highlighted in present study point out changes in zinc metabolism which contributes to an oxidative stress manifestation in patients with type 2 diabetes mellitus.

3. Low serum manganese is more common among diabetes mellitus type 2.

4. When compared to nondiabetic healthy control patients found that type 2 diabetics had low serum chromium levels .

5. As early in the development of DMT2 plasma phosphate levels may be normal or even low, these deregulations may be difficult to distinguish .

6. Depending on the results obtained , the atomic absorption technique is more sensitive , specific and superior for trace elements determination .

Recommendations:

1. Zinc , manganese ,and chromium preferred to be measured for all patients with diabetes mellitus type 2.

2.Future study (therapeutic trial) to evaluate the benefit supplement of trace elements could be helpful in the treatment of diabetes mellitus type2.

3. Completely rely on the atomic absorption spectrophotometer technique in trace elements determination.

4. Determine serum phosphate by spectrophotometric method, This method is much simple, compared with the other developed method .

5. Estimation of trace elements might be good value in caring for DM2with CKD.

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كذلك عمدت الدراسة الى قياس مصل الزنك بطريقتين الامتصاص الذري و المطياف الضوئي .اظهرت النتائج ان تقنية الامتصاص الذري اكثر حساسية وتحديدا و تفوقا في تقدير العناصر النزرة.

أستنتجت الدراسة إلى وجود صلة أكبر بين مؤشر كتلة الجسم وظهور مرض السكر.لخصت الدراسة إلى أن المصابين بمرض السكري لديهم مستوى منخفض من الزنك مقارنة بالأصحاء. تشير الأدلة العلمية التي تم إبرازها في هذه المراجعة إلى التغيرات في استقلاب الزنك الذي يساهم في مظهر الإجهاد التأكسدي لدى مرضى السكري من النوع ٢.
وأوضحت الدراسة ان مرضى السكري لديهم مستويات منخضة من المنغنيز والكروم مما يشير الى دور هذه العناصر في استقلاب السكر في الجسم.

الخلاصة

داء السكرمن الأمراض الأيضية التي تتميز بفرط سكر الدم المزمن الناتج عن عيوب في إفراز الأنسولين أو عمل الأنسولين أو كليهما. تعطي الاضطرابات الأيضية في الكربوهيدرات والدهون والبروتينات مؤشرا مهما عن دور الأنسولين .
بينت الدراسة وجود علاقة بين داء السكري والعناصر النزرة واستخدام طرق مختلفة لقياسها وتقديرها ومقارنتها , اوضحت العديد من الدراسات البحثية السابقة ظهرور تغيير في التمثيل الغذائي لهذه المعادن. ان تقوية عمل الأنسولين بواسطة بعض العناصر النزرة مثل الكروم والزنك والمنغنيز والفوسفوروالتي تتضمن الآليات المقترحة لتعزيز عمل الأنسولين بواسطة العناصر النزرة من خلال تنشيط مواقع مستقبلات الأنسولين ، والتي تعمل كعوامل مساعدة أو مكونات لأنظمة الإنزيم والتي تشارك في استقلاب الجلوكوز ، وزيادة حساسية الأنسولين والعمل كمضادات للأكسدة لمنع أكسدة الأنسجة.
هدفت الدراسة إلى تقدير مستويات مصل الزنك والفوسفور والكروم والمنغنيز في مرضى السكري من النوع الثاني ومقارنتها مع مجموعة الاصحاء وتقييم تأثير هذه المستويات على شدة المرض وتطوره ، وتحديد الارتباط بينهم.
لتحقيق هذا الهدف تم جمع ١٨٠ عينةً حضروا إلى مستشفى الصويرة في الفترة ما بين أكتوبر إلى ديسمبر ٢٠٢١. تراوحت أعمار الأفراد بين ٣٥ و ٦٩ سنة, حيث شملت العينة (٩٠) مريضًا بالسكري من النوع الثاني وكانت (٥٦) اناث و (٣٤) ذكور, و(٩٠) من الاصحاء ، تم حساب حجم العينة من خلال المعادلة الخاصة بذالك والتي تتوافق مع اهداف قسم صحة المجتمع بالكلية.
تم قياس لكل عينات الامصال سكر الدم الصائم واليورياوالكرياتنين والزنك والفسفور بواسطة مقياس الطيف الضوئي وقياس الزنك و الكروم و المنغنيز بواسطة مقياس طيف الامتصاص الذري حيث وجد:
هنالك فرق معنوي بالسكر لمرضى السكري و معدل طبيعي لليوريا و الكرياتنين لاثبات عمل الكلية الطبيعي للمرضى مع مراعات ان جميع العينات هي لاشخاص لديهم زيادة بالوزن و سمنة.
بينت الدراسة ان قيم تراكيز العناصر النزرة (الزنك و الكروم و المنغنيز) منخفضة انخفاض كبيرعن مستواهم الطبيعي لمرضى السكري من النوع الثاني مقارنة بالاصحاء.
وكذالك بينت الدراسة عدم وجود فروق ذات دلالة احصائية بين مرضى السكري و الاصحاء فيما يتعلق بتركيز الفسفور بالدم .

جمهورية العراق

وزارة التعليم العالي و البحث العلمي

جامعة بابل كلية الطب

تقدير نسبة الكروم و الزنك و الفسفور و المنغنيز في مرضى السكري النوع الثاني بتقنيات مختلفة

رسالة

مقدمة الى كلية الطب في جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير في العلوم الكيمياء الحياتية السريرية

من قبل

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