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Estimation the level of the copper in patients with diabetes mellitus among the Iraqi diabetic patients

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بسم الله الرحمن الرحيم

﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ﴾

صدق الله العظيم

سورة المجادلة

الاية ١١

Dedication

I dedicate my graduation:

To the oppressed martyr in her memory, the Lady of the Women of the Worlds… Lady Fatima Al-Zahra Peace be upon her.

To the one who faced the exhaustion of studying with me day after day and calling to ensure my safe arrival to the university,the source of my strength and success,my hero… My Dear Father

To the one who stayed up late nights for my comfort and awoke at dawn to food me and pray for me…My Beloved Mother

To those who shared my struggles with studying and sleepless nights, and who were the most helpful in my journey… My Dear Sisters and Brothers

Finally, to all the family and friends…

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Thank you especially to the pharmasict and friends who helped and guided me. Finally, I am grateful to my dear father, mother, and all of my family members for enabled me to complete this study. Their love and support have given me strength and motivation.

Aim of study

This study was aimed to evaluate the levels of copper concentrations in diabetic patients and compared with controlled people

Abstract

Diabetes Mellitus (DM) is characterized by elevated blood glucose levels (hyperglycemia). It can occur due to impaired secretion or action of the hormone insulin, which is produced by pancreatic beta-cells to promote the entry of glucose into the cells. It is known that hyperglycemia has an important role in the production of reactive oxygen species in all types of DM, in this study spectrophotometry method was used to determination copper metal, the results showed Different levels of Cu metals comparing between diabetic group with the control group. An increased copper levels in DM group in comparing control group where its found (128.4-149) for diabetic group while the level of copper (83.3-94.9) for the control group

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Abbreviations	**The meaning**
HbA1c	test—is a simple blood test that measures your average blood sugar levels over thepast 3 months.
GLUT1	Glucose transporter type 1
GLUT4	Glucose transporter type 4
ROS	reactive oxygen species
NADPH	nicotinamide adenine dinucleotide phosphate
DM	Diabetic mellites
CP	ceruloplasmin

Chapter One Introduction

1. Introduction

1.1 Diabetes mellitus:

Diabetes mellitus is a metabolic disease characterized by high blood glucose levels and various symptoms that persist over time. It is a prevalent condition globally, with a possibility of doubling in the coming years[1].

It develops when the pancreas doesn't make enough insulin or any at all, or when the body isn't responding to the effects of insulin properly. Diabetes affects people of all ages. Most forms of diabetes are chronic (lifelong), and all forms are manageable with medications and/or lifestyle changes. [2].

Several pathogenetic processes are involved in the development of diabetes.These include processes which destroy the beta cells of the pancreas withconsequent insulin deficiency, and others that result in resistance to insulinaction. The abnormalities of carbohydrate, fat and protein metabolism are dueto deficient action of insulin on target tissues resulting from insensitivity or lack of insulin. [3]

The technical name for diabetes is diabetes mellitus. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness,nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation[4].

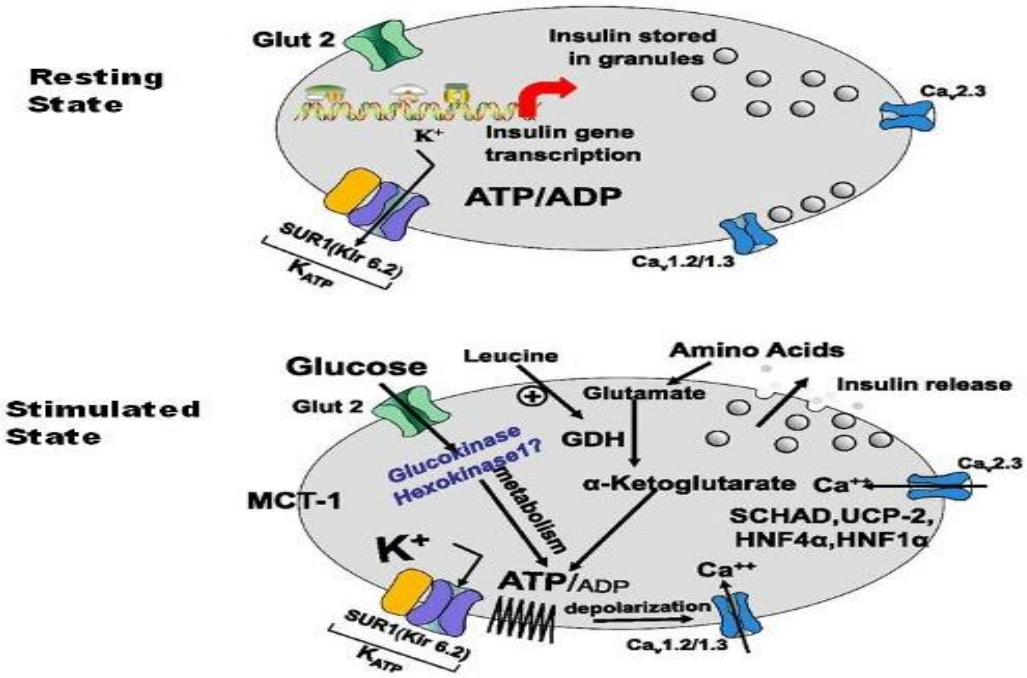
1.2 Pathophysiology:

Insulin is synthesized as preproinsulin in the ribosomes of rough endoplasmic reticulum. The preproinsulin is then cleaved to proinsulin that is transported to the Golgi apparatus where it is packaged into secretory granules. Most of the proinsulin is cleaved into equimolar amounts of insulin and connecting (or C)-peptide in the secretory granules. Because the C-peptide sequence differs from that of insulin, and because, unlike insulin, it is not extracted by the liver, it is possible to estimate β-cell insulin secretion by measuring C-peptide, even in the presence of insulin antibodies resulting from insulin replacement therapy that impair the ability to measure insulin directly. Similarly, because C-peptide

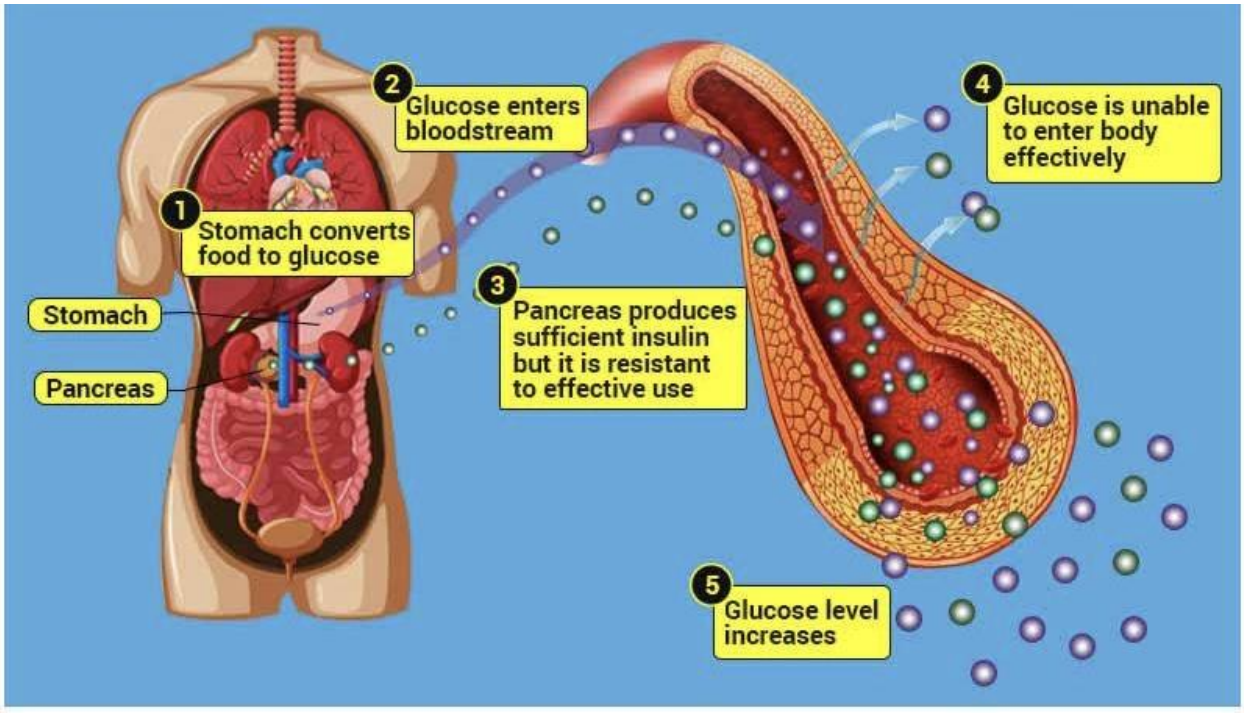
is an index of endogenous insulin secretion, and because C-peptide is not extracted by the liver, the ratio of C-peptide: insulin should exceed 1; when it is less than 1, implying a high insulin value, exogenous insulin may have been used. This has diagnostic and forensic utility in diagnosing causes of hypoglycemia. [5]

Glucose is a major regulator of insulin secretion

When extracellular fluid glucose concentrations rise after a meal, glucose is taken up by the ß cells via glucose transporters, GLUT2 and GLUT1. Glucose is then phosphorylated into glucose-6-phosphate by islet specific glucokinase and metabolized, thereby increasing cellular ATP concentrations. The rise in ATP raises the resting ratio of ATP:ADP, that closes ATP dependent potassium channels (K-ATP) in the β-cell membrane, resulting in accumulation of intracellular potassium, causing membrane depolarization and influx of calcium via a voltage gated calcium channel. The rise in intracellular free calcium in ß-cells promotes margination of the secretory granules, their fusion with the cell membrane, and release of cell contents which include insulin into the extracellular space. An immediately releasable pool of insulin granules adjacent to the plasma membrane is responsible for an acute (first phase) insulin response; with ongoing stimulation, a pool of granules in the interior of the cell is mobilized and released as the "second phase" response. Amino acids also stimulate insulin release by a similar mechanism that involves the enzyme glutamate dehydrogenase which enables metabolism and ATP production by certain amino acids. Defects in the genes regulating these processes may result in diabetes if the K-ATP channel is prevented from closing normally (activating mutations) or syndromes of hyperinsulinemic hypoglycemia if the K-ATP channel is prevented from opening (inactivating mutations [6]



Stimulation of insulin(1.1)



Insulin resistant (1.2)

1.3 Types of diabetes

There are three main types of diabetes: type 1, type 2, and gestational diabetes

1.3.1 Type 1 diabetes:

Type 1 diabetes is a chronic illness characterized by the body's inability to produce insulin due to the autoimmune destruction of the beta cells in the pancreas. Although onset frequently occurs in childhood, the disease can also develop in adults (the body attacks itself by mistake). This reaction stops your body from making insulin. Approximately 5-10% of the people who have diabetes have type1.

Symptoms of type 1 diabetes often develop quickly.If you have type 1 diabetes, you'll need to

take insulin every day to survive. Currently, no one knows how to prevent

type 1 diabetes

Signs and symptoms

The classic symptoms of type 1 diabetes are as follows:

- Polyuria
- Polydipsia
- Polyphagia
- Unexplained weight loss.[7]

1.3.2 Type 2 diabetes:

is characterized by a lack of sensitivity of target organs to

insulin. In type 2 diabetes, the pancreas retains some β -cell function, but insulin

secretion is insufficient to maintain glucose homeostasis in the face of increasing

peripheral insulin resistance. The β-cell mass may gradually decline over time in

type 2 diabetes. In contrast to patients with type 1 diabetes, those with type 2

diabetes are often obese. Obesity contributes to insulin resistance, which is considered the major underlying defect of type 2 diabetes. With type 2 diabetes, your body doesn't use insulin well and can't keep blood sugar at normal levels. About 90-95% of people with diabetes have

type 2. It develops over many years and is usually diagnosed in adults.

Symptoms of Type 2 diabetes

1-Increased thirst.

2-nogangis

3-Increased hunger.

4-Fatigue.

5-Slow healing.

6-Numbness in hands or feet.

7-Blurred vision.

8-Frequent urination.

9-Dry skin.

So it's important to get your blood sugar tested if you're at risk and can be regulated by oral therapy

Type 2 diabetes can be prevented or delayed with healthy lifestyle changes,

such as:

• Losing weight.

• Eating healthy food.

• Being active[8]

1.3.3 Gestational Diabetes:

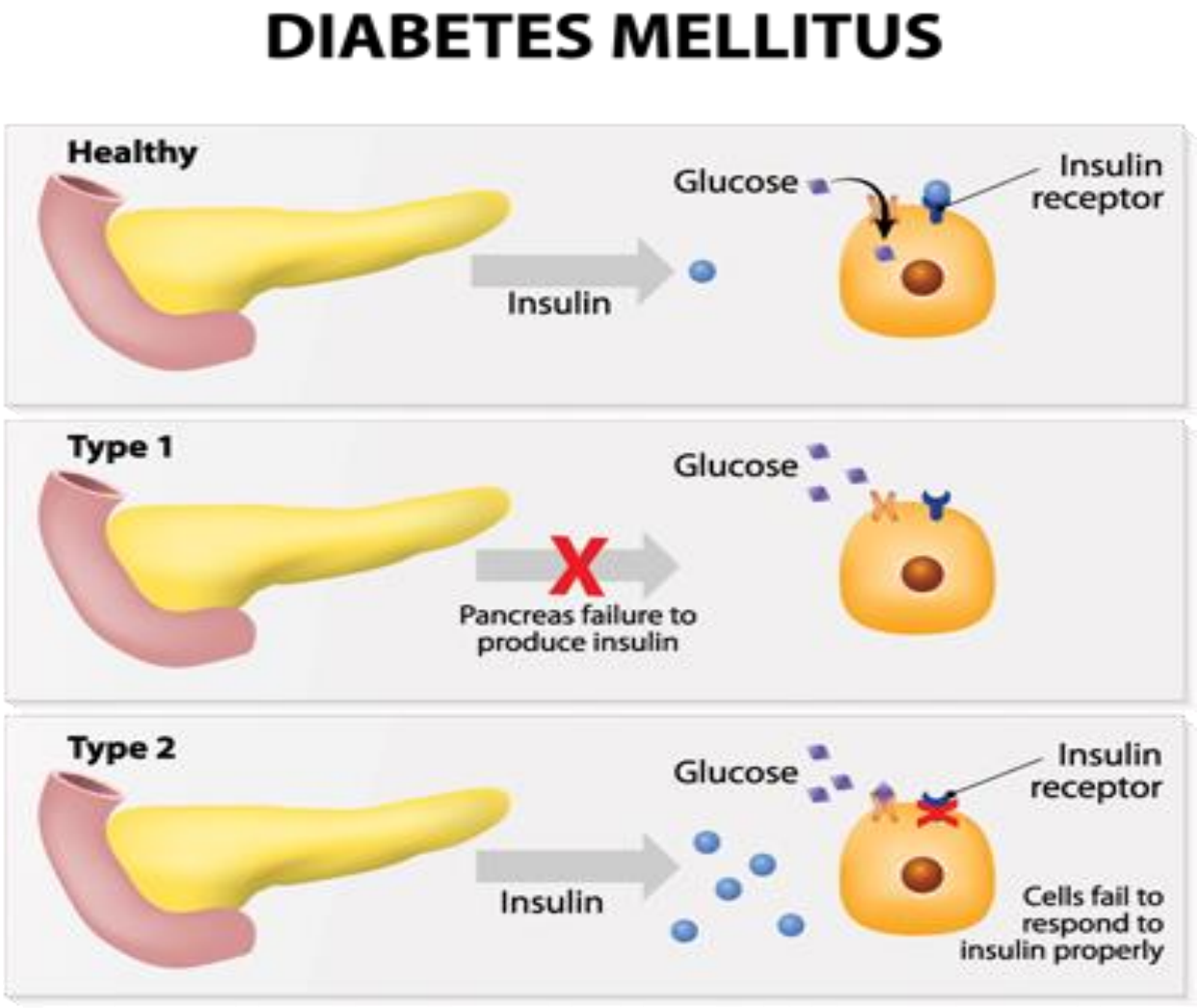
Gestational diabetes develops in pregnant women who have never had

diabetes. If you have gestational diabetes, your baby could be at higher risk

for health problems. Gestational diabetes goes away after your baby is born.

However, it increases your risk for type 2 diabetes later in life. Your baby is

more likely to have obesity as a child or teen and develop type 2 diabetes later in life.[9]



Types of diabetes mellitus(1-3)

1.3.4 Risk factors of diabetes mellitus:

Non-modifiable risk factors:

1-Family history

2-Race or ethnic background.

3-Age

3-Gestational diabetes

Modifiable risk factors:

1-Weight

2-Physical activity

3-Blood pressure

4-Smoking. [10]

1.4 Copper:

Cu is the third most abundant trace element in the human body

Copper, an essential mineral, is naturally present in some foods and is available as a dietary supplement. It is a cofactor for several enzymes (known as cuproenzymes) involved in energy production, iron metabolism, neuropeptide activation, connective tissue synthesis, and neurotransmitter synthesis .One abundant cuproenzyme is ceruloplasmin (CP), which plays a role in iron metabolism and carries more than 95% of the total copper in healthy human plasma [11]. Copper is also involved in many physiologic processes, such as angiogenesis; neurohormone

A wide variety of plant and animal foods contain copper, and the average human diet provides approximately 1,400 mcg/day for men and 1,100 mcg/day for women that is primarily absorbed in the upper small intestine. Almost two-thirds of the body's copper is located in the skeleton and muscle.

Only small amounts of copper are typically stored in the body, and the average adult has a total body content of 70-153 mcg/dl in serum .Most copper is excreted in bile, and a small amount is excreted in urine. Total fecal losses of copper of biliary origin and nonabsorbed dietary copper are about 1 mg/day

[12]. Copper levels in the body are homeostatically maintained by copper absorption from the intestine and copper release by the liver into bile to provide protection from copper deficiency and toxicity [13].

Human studies typically measure copper and cuproenzyme activity in plasma and blood cells because individuals with known copper deficiency often have low blood levels of copper and CP [14]. However, plasma CP and copper levels can be influenced by other factors, such as estrogen status, pregnancy, infection, inflammation, and some cancers [15]. Normal serum concentrations are 10–25 mcmol/L for copper and 180–400 mg/L for CP [16].

1.4.1 Functions of copper:

Copper is an essential nutrient for the body1. It plays a crucial part in fundamental biochemical pathways of glucose and cholesterol metabolism2. Copper facilitates several processes of energy generation, collagen protein production, bone formation and coordinates with iron, to ensure healthy red blood cell synthesis. It helps maintain healthy bones, blood vessels, nerves, and immune function, and it contributes to iron absorption. Copper is also involved in the production of brain neurotransmitters. [17]

1.4.2 Sources of Copper in Food

The richest dietary copper sources include shellfish, seeds and nuts, organ meats, wheat-bran cereals, whole-grain products, and chocolate [18]. The absorption of copper is strongly influenced by the amount of copper in the diet; bioavailability ranges from The human gastrointestinal system can absorb 30-40% of ingested copper from the typical diets consumed . [19].

Tap water and other beverages can also be sources of copper, although the amount of copper in these liquids varies by source (ranging from 0.0005 mg/L to 1 mg/L) [20].

1.4.3 Dietary supplements:

Copper is available in dietary supplements containing only copper, in supplements containing copper in combination with other ingredients, and in many multivitamin/mineral products [21]. These supplements contain many different forms of copper, including cupric oxide, cupric sulfate, copper amino acid chelates, and copper gluconate. To date, no studies have compared the

bioavailability of copper from these and other forms [22]. The amount of copper in dietary supplements typically ranges from a few micrograms to 15 mg [23] [24]

1.4.4 Copper deficiency :

Copper deficiency is quite rare as we need only trace amounts and most people get enough from a diet. However, it might be worth investigating in certain cases, such as hypothyroidism, anemia, poor immune function, and hair loss. [25] [26]

Copper deficiency causes:

1- Not consuming enough copper due to a poor diet or malabsorption

2- Consuming too much zinc, which competes with copper for absorption

3- Having an inherited disorder, such as Menkes' disease, that affects copper metabolism

4- Having surgeries affecting the digestive tract, such as gastric bypass, gastrectomy, or esophagectomy

5- Losing excessive copper through urine, sweat, or bleeding[27]

1.4.5 Copper elevation:

a buildup of too much copper is serious. It can result in brain damage, liver failure, or death if it is not treated and the most causes of it are :

1- A genetic abnormality that stops the body from getting rid of copper (Wilson disease)

2- Consuming too much copper from food, water, or supplements

3- Having a liver disease such as biliary cirrhosis

4- Having a condition that causes excess iron absorption such as hemochromatosis [28]

1.5 Copper and diabetes :

Cu is known as the cause of many diseases and plays an important role in the peroxidation mechanisms. Impaired balance in the process leads to an increased oxidative damage in tissue and ultimately to the progress of diabetes and its complications[29]

Copper plays a significant role in the context of diabetes. Research indicates that copper intake levels can influence the development and management of type 2 diabetes mellitus (T2DM) due to its dual action as both an anti-oxidant at adequate levels and as a pro-oxidant in excessive levels ,anti-oxidants are substances that inhibit oxidation.[30]. Oxidation is a chemical process that results in the transfer of electrons—it always occurs along with a reduction—one substance is oxidized while another is reduced. These are termed "redox reactions". In the body, these types of reactions are occurring all the time producing end products that are known as free radicals. Free radicals are highly reactive substances and can bind to DNA and proteins, damaging them permanently and causing cell, tissue and organ damage. In many different chronic diseases, including diabetes, the levels of free radicals overcome the body's ability to soak or sop them up. The high level of antioxidants leads to a condition in the cells, tissues and organs known as oxidative stress. [31]

oxidative stress is strongly associated with diabetes—and particularly with the complications of diabetes.The use of antioxidants—either from food sources or as a supplement may help prevent or potentially reverse damage from oxidative stress is by reducing damage to the endothelium—this is the layer of cells that line blood vessels. [32]

Chapter Two
Practical part

2.1 Method:

Fifteen samples from male and female of diabetic paitents between age (27-38) were collected in Gel Tube from Marjan Hospital and measured the concentration of Cu in laboratory by using spectrophotometer device

Fifteen samples of controlled male and female between age (26-37) were collected and measured and compered with DM paitents.

2.2 principle

Determination of copper concentration (mg/di)

The principle of this method depend on reaction between copper with 4(3,5 di bromo -2-pyridylazo to form -N-ethyl- sulfopropylaniline achelate complex this increase the absorbance can be measured and its proportional to the concentration oΔf total copper in the sample .

Blank	Standard	sample	
Reagent	1.0ml	1.0ml	1.0ml
Standard	……..	50µl	……..
Sample	……	…….	50µl

Mix and incubate for 5 minutes at 37°c measure the absorbance of the sample As and of the standard Ast against the reagent blank ARBL

ΔAs=As-ARBL

ΔAstd=Astd-ARBL

Calculation

Serum copper conc. (µg/dl)= (ΔAs/ΔAstd)*100

Capter Three Result and discussion

3.1 Result and discution:

Copper plays a significant role in insulin production and glucose metabolism in individuals with diabetes. Studies have shown that copper imbalance can lead to oxidative stress, impacting insulin secretion[33]. Furthermore, an imbalance in copper levels can influence the progression of complications in diabetes, such as Diabetic Kidney Disease (DKD) [34]. Research indicates that higher copper concentrations are found in individuals with diabetes compared to healthy individuals, suggesting a potential link between copper levels and diabetes[35] Overall, maintaining adequate copper levels is crucial for proper insulin function and glucose metabolism in individuals with diabetes.[36]

Micronutrients like copper play an essential role in regeneration, for coping with oxidative stress and for an adequate immune response. Hence, this element are essential for maintaining health throughout life. Micronutrients can cause diseases through deficiency, imbalance, or toxicity. Studies have shown that elevated copper may be a contributing factor in many inflammatory conditions[37]

In table (1-1),fig (1-1) there is a significant increase in the cupper level in the patient with diabetic mellitus in compared with control group.

Table (1-1) the level of the copper in patients with diabetes mellitus compared with control group

Characteristic	Diabetes mellitus group n=15	Control Group n=15	P-value
Age(years)			
Mean± SD	**32.7 ± 3.42**	**30.9±4.41**	***p* >0.05**
Range	**27-38**	**26 -37**	
Copper (mg/dl)			
Mean± SD	**136.56 ± 8.61**	**89.53 ±4.89**	***p* <0.05**
Range	**128 .4 -149**	**83.3 - 94.9**	

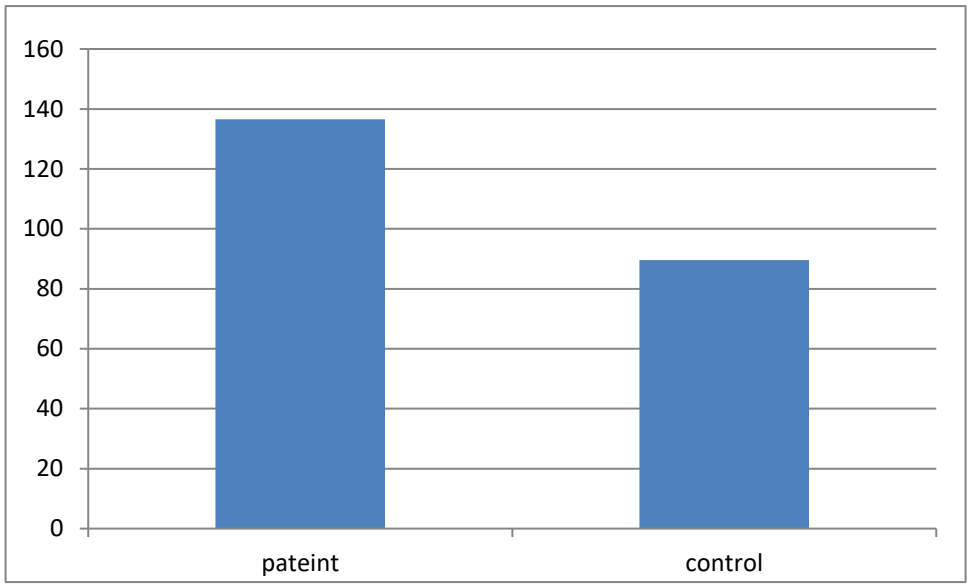


Fig (1-1) the level of the copper in patients with diabetes mellitus compared with control group

Diabetes may elevate copper levels, potentially contributing to oxidative stress in diabetic. Abnormal copper levels are associated with disease progression and complications in diabetic patients. The excess of copper (Cu) levels are associated with an increased level of oxidative stress, which may aggravate the microvascular lesions in diabetes mellitus. Several studies have revealed a significantly increased Cu levels in diabetic patients in compared with controls. These abnormalities are correlated with the duration of diabetes and higher levels of HbA1C. Multiple pathological mechanisms are proposed to explain these changes Increased levels of free Cu ions may be attributed to glycation and the release of Cu ions from the Cu-binding sites of proteins. selective Cu chelators may be useful to alleviate oxidative stress and prevent DR progression [38].

3.2 Conclusions:

1-diabetes mellitus cause raising in blood copper level in compard with control

2-Oxidative stress can be increased due to increasing in Oxygen Reactive Species by increasing in Glucose level.

3.3 Recommendations:

Defective copper regulation is implicated as a causative mechanism of organ damage in diabetes.

1- Treatment with Triethylenetetramine(TETA) which acts as a highly selective divalent copper (Cu(II)) chelator that works to promote urinary copper excretion .It prevents or reverses diabetic copper overload, thereby suppressing oxidative stress. .[39]

2-since zinc acts as anti-oxidant, and there is competitive absorption between it and copper, so using zinc supplement is beneficial in reducing the oxidative stress of copper

3-Restricte the use of food rich in copper.

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