

Estimation and Evaluation of (Uric Acid, Glutathione, Ceroplasmin) and the Trace Elements (Iron, Copper, Zinc) Levels in Type_2 Diabetic Patients in Basrah Governorate-Iraq

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ABSTRACT

Type 2 diabetes occurs mainly as a result of two interrelated problems Cells in the muscles, fat, and liver become insulin resistant, because the cells do not react in a normal way to insulin, they do not absorb enough sugar, and thus the pancreas becomes unable to secrete an adequate amount of insulin to control blood sugar levels. Since antioxidants and trace elements have a role in the treatment of diabetes by defending beta cells against oxidative stress, so the current research was conducted to find out the levels of non-enzymatic antioxidants (Uric acid, Glutathione, Ceruloplasmin) and some trace elements (Iron, Copper, Zinc) that act as antioxidants in serum of type 2 diabetic patients compared to healthy subjects in Basrah Governorate-Iraq by age. The number of patients in the study was (50) patients (26 males-24 females) and healthy subjects (43), including (11 males-32 females). It was found that there was a significant decrease at the level of significance ($p < 0.001$) in the levels of each of (Glutathione, Ceruloplasmin, Iron and Zinc) in patients and healthy subjects and this decrease increased with age, while there was a significant increase at the level of significance ($p < 0.001$) in the levels of (Uric acid and Copper) at patients and healthy subjects with age.

Keywords: ceruloplasmin, copper, glutathione, iron, type II diabetes, uric acid, zinc.

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I. INTRODUCTION

Type 2 diabetes also known as non-insulin dependent diabetes, is the most common metabolic disorder worldwide and occurs as a result of a deficiency in insulin secretion resulting from a defect in pancreatic beta cells and insulin resistance [1]. This type affects adults and represents about 90% of all cases of diabetes [2]. In 2015, there were 5 million deaths due to diabetes worldwide, and blood clots are the main cause of death, as about two-thirds of patients with type 2 diabetes die as a result of clots [3]. Oxidative stress is the main cause of chronic genetic diseases, including diabetes, as it increases the production of oxygen free radicals ROS that cause damage to pancreatic beta cells, leading to type 2 diabetes [4]. Since antioxidants have a role in the treatment of diabetes by defending beta cells against oxidative stress resulting from programmed cell death [5].

It was found that uric acid, glutathione, and ceruloplasmin are powerful antioxidants, Uric acid is the final product of purine metabolism in humans it has the ability to protect against oxidative damage by donating an electron, it inhibits peroxy radical and hydroxyl radical, in addition, it prevents oxidation of low-density lipoproteins, the ability to act as a chelator for metal ions such as iron and copper by converting them into weakly reactive forms that are unable to catalyze free radical reactions [6], [7]. The normal rate of

uric acid in the body is about (2–7 mg/dL) for males and (2–6 mg/dL) for females [8].

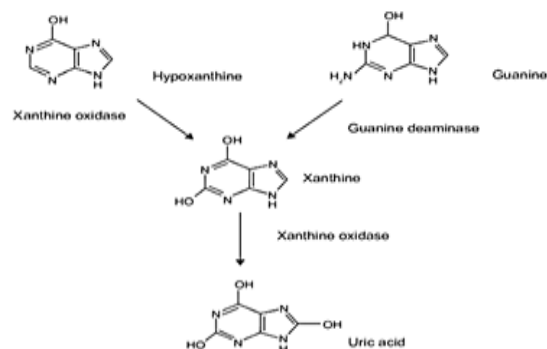


Fig. 1: Equations for the formation of uric acid [9].

As for glutathione, it is a peptide consisting of three amino acids, glycine, cysteine, and glutamine it is considered one of the most effective low-molecular-weight antioxidants, as the thiol group (-SH) of cysteine participates in redox reactions, which is one of the most important functions of glutathione it protects cells from oxidative stress and gets rid of Directly from oxidants such as super anion, hydroxyl radical, nitric oxide radical, and detoxifiers such as lipid hydroxides and peroxides [10]. The normal level of glutathione in the human body is about (0.1–10 mmol), [11].

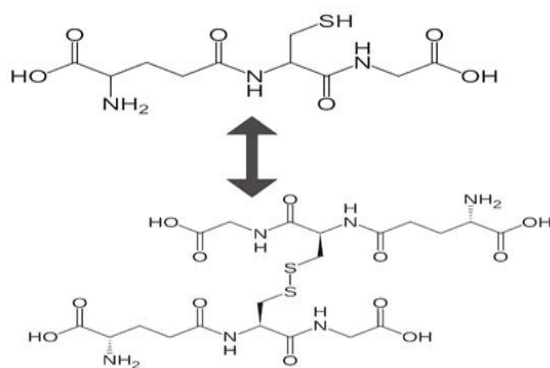


Fig. 2: Chemical structures of reduced and oxidized glutathione[12].

While ceruloplasmin is a glycoprotein consisting of 1046 amino acids and has many biological functions, it is a catalyst in oxidation-reduction reactions it can oxidize iron Fe^{+2} to Fe^{+3} and this helps to bind iron to transferrin and acts as an antioxidant by donating free copper ions that stimulate the formation of oxygen free radicals and are reduced oxygen directly into water[13], [14]. The normal level of ceruloplasmin in the blood is about (25 mg/dl), [15]. Also, trace elements have very important roles in the human body, the biological activities of (Fe, Cu and Zn) are closely linked to the presence of unpaired electrons that allow them to participate in oxidation and reduction reactions [16]. Iron is one of the most abundant metals in the human body and plays a major role in basic chemical activities such as formation of oxygen radicals and electron transport It exists in two states, the second valence iron Fe^{+2} , and the trivalent iron Fe^{+3} , as it participates directly as a acceptor or donor in electron transfer reactions [17]. The normal level of iron in the body is about (40–50 mg) per kilogram of body weight [18]. While copper is the third largest element in the body than iron and zinc [19]. It forms part of many copper-dependent enzymes and proteins such as tyrosinase, cytochrome oxidase, superoxide dismutase and ceruloplasmin, and the importance of copper is due to its ability to accept and donate electrons easily, as copper in the body converts between Cu^{+1} and Cu^{+2} , and the majority of copper in the body is the second form of oxidation this explains its important role in the reactions of reducing oxidative stress and free radicals in the organism [17]. The normal range of copper in the human body is about (50–80mg), [19]. Zinc is one of the most rare and abundant elements in the human body, it has an important role in human growth and development during pregnancy, childhood and adolescence, it is involved in the formation of more than 300 enzymes and is found in the cytoplasm of the cell, it participates in the synthesis of protein, nucleic acid, carbohydrates, alcohol metabolism and protein metabolism [17]. Zinc also plays a major role in the storage and secretion of insulin, which increases the absorption of glucose, and low zinc negatively affects the ability of beta cells to produce and secrete insulin [19]. And zinc is a structural part of the main antioxidant enzymes such as superoxide dismutase (SOD), and zinc deficiency weakens its composition, which leads to increased oxidative stress, and the normal rate of zinc in the human body is about (84–159 $\mu\text{g/dl}$), [20].

Our current study aims to estimate the levels of non-enzymatic antioxidants (Uric acid, Glutathione,

Ceruloplasmin) and some trace elements (Iron, Copper, Zinc) in the serum of the study groups with age.

II. MATERIALS AND METHODS

A. Blood Sampling

All patients' blood samples were taken from the Specialized Hospital for Diseases and Surgery of the Digestive System and Liver in Basrah Governorate-Iraq. The study included (50) sick cases, including (26 males-24 females) and (43) healthy cases (11 males-32 females) their information was recorded according to a questionnaire that included (age, gender, family history, duration of illness, and housing).

B. Preparation of Blood Samples

Blood samples were collected by drawing venous blood (5 ml) with a medical syringe, then placed in tubes that do not contain anticoagulant, then the tubes were left for (10 minutes) until the blood coagulated, then placed in a centrifuge for (15 minutes) to separate the serum from the blood components. Then, the serum was transferred into small tubes and kept at a temperature of (-4°C) until the required laboratory tests were performed.

C. Biochemical Measurements

The concentration of uric acid in the serum of patients and healthy subjects was diagnosed based on the colorimetric method using the diagnostic kit prepared by the French company (BOIOLABO). As for the concentration of glutathione in the blood serum of patients and healthy people, it was diagnosed using the modified method by the researchers [21] the method of diagnosis included the reaction of a solution of Mann (5-5-di thio bis (2- Nitro benzoic acid) with glutathione, as it reacts quickly and is reduced by the sulfhydryl group (SH group) of glutathione and forms a colored complex whose absorbance is measured at a wavelength (412nm), [21].

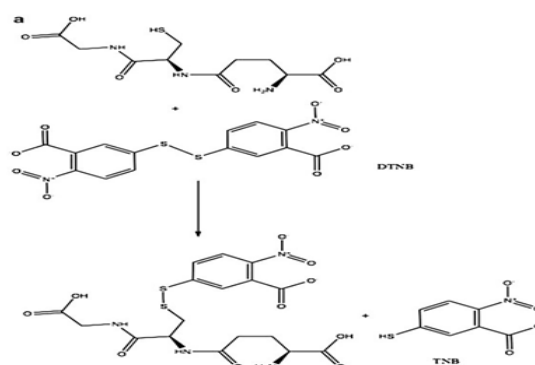


Fig. 3: Formation of the chromatography complex from the reaction of glutathione with DTNB reagent[22].

The concentration of ceruloplasmin in the blood serum of patients and healthy people was diagnosed based on the method modified by the researchers (Menden et.al), the diagnostic method depends on the effectiveness of ceruloplasmin on the oxidation of (P-phenylendiamine) colorless to a blue-violet solution, and its absorbance was measured at a wavelength (525nm), [23]. The concentrations of the elements (iron, copper, zinc) in the blood serum of

patients and healthy people were diagnosed based on the colorimetric method using the diagnostic kit prepared by different companies.

D. Statistical Analysis

Statistical operations were carried out in the SPSS statistical program version (24) to compare between the study groups using one-way analysis of variance (ANOVA) in the least significant way to know the effect of significant coefficients and to determine the presence of significant differences at the value of ($P<0.05$) and high significant differences at ($P<0.001$) As well as the use of statistical analysis to extract the values of the average mean and standard deviation of the characteristics of diabetic patients and healthy subjects.

III. RESULTS

The results of our current study in Table I and Fig. 2, 3, 4, 6 showed a significant decrease at the level of significance ($P<0.001$) in the levels of (Glutathione, Ceruloplasmin, Iron, Zinc) In the serum of both type 2 diabetic patients and healthy subjects with age. While there was a significant increase at the level of significance ($P<0.001$) in the levels of each of (Uric acid and Copper) in the blood serum of both diabetic patients and healthy people with advancing age, as shown in Table I and Fig. 1, 5.

TABLE I: CONCENTRATIONS OF EACH OF THE NON-ENZYMATIC ANTIOXIDANTS (URIC ACID, GSH, CP) AND SOME TRACE ELEMENTS (Fe, Cu, Zn) IN THE BLOOD SERUM OF DIABETIC PATIENTS AND HEALTHY SUBJECTS BY AGE GROUP FACTOR

Parameters		Concentration values		
		Age (30–40) year	Age (41–50) year	Age (>50) year
Uric acid mg/dl	Patients	9.50±3.89***	10.31±3.09***	10.74±3.52***
	Control	5.30±1.15	5.81±1.36	6.09±1.80
GSH μ mol/l	Patients	0.51±0.34***	0.49±0.06***	0.40±0.28***
	Control	4.95±1.21	4.59±2.63	3.29±1.04
CP mg/dl	Patients	41.87±18.75***	42.64±23.41***	29.03±10.26 NS
	Control	22.47±7.80	23.44±10.94	20.43±8.72
Fe μ g/dl	Patients	275.12±34.51***	209.67±55.56***	192.83±34.17***
	Control	152.41±43.09	142.80±50.64	135.87±56.01
Cu μ g/dl	Patients	227.37±65.01***	236.68±69.13***	243.71±71.68***
	Control	91.27±40.31	116.58±61.72	152.29±28.05
Zn μ g/dl	Patients	64.66±22.22***	52.68±19.32***	51.20±20.92***
	Control	146.90±56.33	127.76±70.02	147.96±61.86

Concentration values were expressed as mean±SD *** $P<0.001$, ** $P<0.01$, * $P<0.05$. NS: Non-significant.

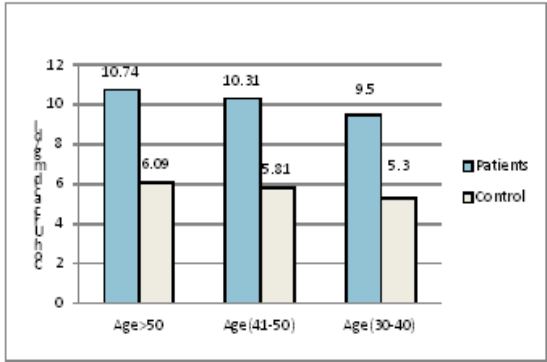


Fig. 1. Uric acid concentration in the blood serum of diabetic patients and healthy subjects according to age factor.

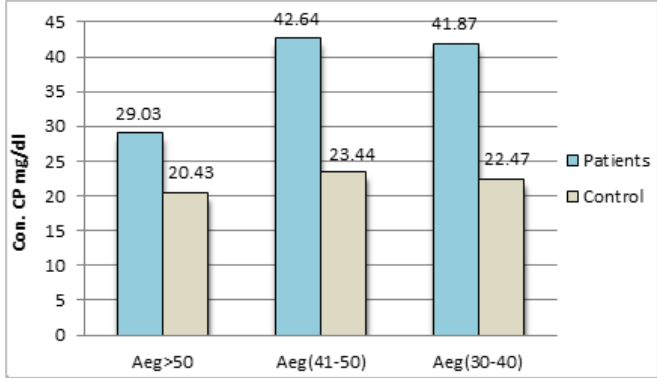


Fig. 3. Serum ceruloplasmin CP concentration in diabetic patients and healthy subjects by age factor.

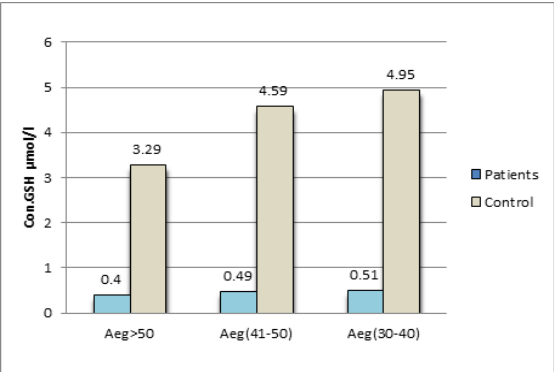


Fig. 2. Average concentration of glutathione (GSH) in the blood serum of diabetic patients and healthy subjects by age factor.

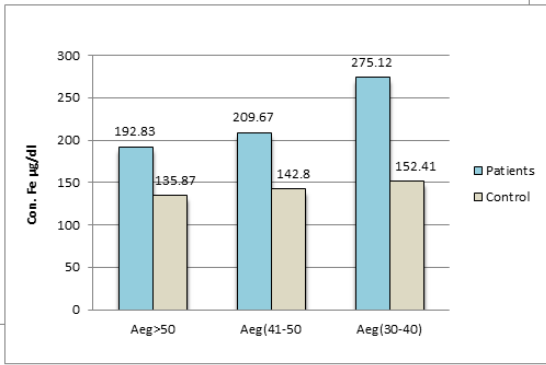


Fig. 4. Average concentration of iron (Fe) in the blood serum of diabetic patients and healthy subjects according to the age factor.

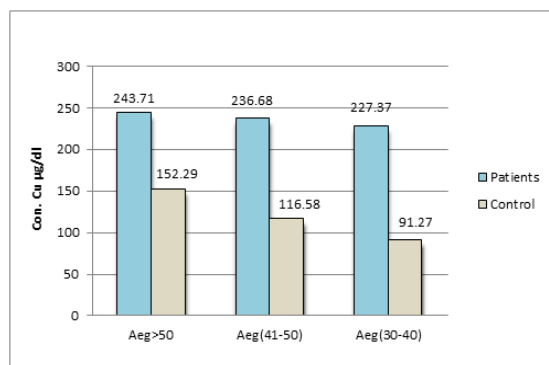


Fig. 5. Average concentration of iron (Fe) in the blood serum of diabetic patients and healthy subjects according to the age factor.

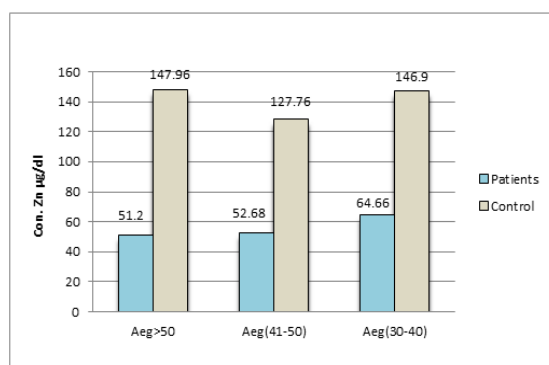


Fig. 6. The mean concentration of Zn in the blood serum of diabetic patients and healthy subjects, according to the age factor.

IV. DISCUSSION

The results of our current study showed a significant decrease in glutathione levels in patients and healthy subjects with age, because oxidative stress increases in old age, but it is still unclear whether the increase in oxidative stress during aging results from a decrease in antioxidant levels or an increase in the generation of free radicals. Glutathione is a major component of the antioxidant system in general, however, many previous studies have shown that the reduced form of glutathione, GSH, decreases during aging. This is consistent with another study conducted on mice, and their ages varied between (6–28) months, they noticed that glutathione concentrations in the liver were significantly lower in older mice [24]. The results of our study also showed a significant decrease in ceruloplasmin levels in both patients and healthy subjects with age, this indicates that the activity of ceruloplasmin decreases in human blood with age, these results are consistent with the results of another study conducted on 120 people whose ages were between (45–102) years they studied the effect of age on the activity of ceruloplasmin on them and concluded that the decrease in the activity of ceruloplasmin depends on age has a negative effect on the antioxidant functions in the blood and finally on the aging process itself [25]. While the results of our study showed a significant increase in uric acid levels in both patients and healthy people, because with age, the incidence of type 2 diabetes increased with the increase in uric acid as a result of an increase in insulin resistance [26]. While the results of our study showed a significant decrease in the levels of both iron and zinc, and the reason for the decrease in the elements in elderly

diabetic patients is attributed to because they consume less than the recommended amounts in the diets or as a result of lack of absorption, the decrease in the intake of iron-rich red meat may be a result of a certain diet, therefore, the proportion of iron in them decreases, while as a result of the high excretion of zinc in the urine, which overcomes its absorption, and this leads to a decrease in zinc levels, the results of our study are consistent with the results of another previous study [27]. For this reason, we advise diabetic patients to take nutritional supplements to reduce the complications of diabetes as they age. The results of our study also showed a very significant increase in copper levels in both patients and healthy people with age, due to the increase in blood sugar, which triggers the release of Cu^{+2} binary ions from copper-containing enzymes, and these results are consistent with the results of the research [28].

V. CONCLUSIONS

Since type 2 diabetes affects people aged between (40–70) years, this study was conducted to estimate and evaluate the levels of non-enzymatic antioxidants and some trace elements for three different age groups to know their effect on the development of complications of type 2 diabetes with age, there is a significant decrease in the levels of glutathione, ceruloplasmin, iron and zinc with age, while there was a significant increase in the levels of uric acid and copper in diabetic patients with age, and this indicates that oxidative stress increases in the aging stage and plays a major role in the development of diabetes complications type II with age.

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