Study of serum ceruloplasmin and zinc in type 2 diabetes mellitus

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Abstract

Background and objective: Ceruloplasmin is the major copper-carrying protein in the blood and plays a role in iron metabolism. Zinc is an essential trace element for human health, which activates about 200 enzymes. This study aimed to evaluate the serum ceruloplasmin and zinc and the factor affecting them in type 2 diabetes.

Methods: A case-control study was carried out from November 2018 to June 2019 in collaboration between the biochemistry unit at the College of Medicine and the biochemistry unit at the College of Nursing, Hawler Medical University. The study included 100 type 2 diabetic patients (group 1) and 100 healthy individuals (group 2) to evaluate the serum ceruloplasmin and zinc in both groups.

Results: The mean value of serum ceruloplasmin was significantly (P <0.001) higher in type 2 diabetic patients (55.9 ± 1.26 mg/dL) than that of healthy individuals (36.65 ± 0.71 mg/dL). The mean value of serum zinc was significantly (P <0.001) lower in type 2 diabetic patients (39.19 ± 0.46 ug/dL) compared with controls (65.27 ± 2.5 ug/dL). There was a significant positive correlation between serum ceruloplasmin and the age of diabetic patients (P <0.001). There was a significant negative correlation between serum ceruloplasmin and serum zinc of diabetic patients (P <001). There was no significant correlation between serum ceruloplasmin and body mass index of diabetic patients (P = 0.928).

Conclusion: Based on the findings of the present study, we concluded that high serum ceruloplasmin and low zinc were associated with type 2 diabetics.

Keywords: Serum ceruloplasmin; Age; BMI; Zinc; Type 2 diabetes mellitus.

Introduction

Diabetes mellitus was the 8th leading cause of death, accounting for 4% (1.5 million) of all deaths under the age of 70 in 2012 globally. The most recent data from WHO revealed that 422 million adults are living with diabetes mellitus.[1,2] The disease is caused in most cases by a deficiency or complete lack of the hormone insulin, which is produced in the pancreas, or by an inability of the body to respond appropriately to insulin (i.e., insulin resistance). Both conditions can include chronically elevated blood glucose levels,[3,4] excessive excretion of glucose in the urine, and the accumulation of certain acidic substances in the blood. If not prevented or treated properly, these changes can lead to coma and even death. Other adverse events associated with diabetes include the increased risks of associated complications e.g., heart disease, stroke, kidney failure, accounting for another 2.2 million deaths worldwide.[5,6] Ceruloplasmin is a circulating blue multicopper oxidase that contains >95% of copper in the plasma. Ceruloplasmin is synthesized mainly in the liver as a single chain polypeptide. After incorporating six atoms of copper early in the biosynthetic pathway, it is secreted into the plasma as an α2-glycoprotein.[7] Ceruloplasmin was first described in 1948. The molecular weight of human ceruloplasmin is reported to be

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151kDa.[8] Its precise biological roles are unknown, but may be related to copper transport, iron metabolism,[9] and antioxidant defense.[9] Serum level of ceruloplasmin increases during infection and tissue injury. On the other hand, a decrease of the protein in the plasma is observed in some diseases, such as Wilson's disease.[10] Hereditary hypoceruloplasminemia and hereditary ceruloplasmin deficiency (HCD). HCD is an autosomal recessive disease characterized by neurological abnormalities such as progressive cerebral degeneration, complete ceruloplasmin deficiency, and excessive storage of iron in the systemic organs, such as the liver and brain. In many HCD cases, type 2 diabetes is the first symptom, and 5-20 years later, at ages 40-60 years, the neurological abnormalities occur.[10] An increase in serum ceruloplasmin levels has also been reported in type 2 diabetes.[11] However, it has been reported that blood HbAlc levels, duration of type 2 diabetes, patient age, and the presence or absence of diabetes complications are not major factors influencing its increase.[12] So, the question is what factor increases serum ceruloplasmin in type 2 diabetes? To clarify it, serum ceruloplasmin was measured from a relatively large number of type 2 diabetes patients and confirmed an increase in serum ceruloplasmin levels in type 2 diabetes. Next, it was found that a high blood glucose level is one of the important factors.[12] Trace elements are accepted as essential substances for optimum human health because of their diverse metabolic characteristics and functions. They serve a variety of catalytic, structural, and regulatory functions in which they interact with macromolecules such as enzymes and pro hormones.[13] Direct association of minerals, trace elements, and vitamins in the pathogenesis and natural course of both type 1 and 2 diabetes mellitus has been observed in many research studies. An alteration in the metabolism of these minerals and vitami ns has been demonstrated. Some trace elements act as antioxidants; prevent membrane peroxidation while others act directly on glucose metabolism.[14] Zinc is an essential trace element, is a component of many enzymes, and plays an important role in the maintenance of several tissue functions, including the synthesis, storage, and release of insulin.[15] Zinc plays an important role in glucose metabolism. It has been found to enhance the effectiveness of insulin in vitro, and it has been postulated that its deficiency may aggravate the insulin resistance in non-insulin dependent diabetes mellitus.[16] This study aimed to estimate serum zinc and ceruloplasmin levels in type 2 diabetes mellitus compared with that of healthy individuals and identify the inter-relationship among these components in healthy controls and type 2 diabetes mellitus.

Methods

Subjects and Design of the Study
This study was conducted over a period of eight months, from the 10th of November 2018 to the 30th of June 2019. The subjects included 100 type 2 diabetic patients (35 men and 65 women) as group 1, with the mean age of 53.85 ± 1.171 years, and 100 healthy volunteers (35 men and 65 women) as group 2 with the mean age of 46.42 ± 1.17 years. All the cases in both groups (1 and 2) were non smokers and non alcohol drinkers.

Serum Sampling
Four to six milliliters of venous blood were withdrawn from each individual using a disposable syringe. The samples were obtained from Layla Qasim center specialized for diabetic patients. The samples after half an hour were centrifuged for 10 minutes at 3000 rpm, and the serum was analyzed directly.

Methods

Estimation of the serum ceruloplasmin
Ceruloplasmin, ferro-oxidase, catalyzes the oxidation of some polyamines, and its action on p-phenylene diamine is measured as the amount in serum.[17]

Estimation of the serum zinc

Zinc forms with 2-(5-Brom-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol a red chelate complex. The increase of absorbance can be measured and is proportional to the concentration of total zinc in the sample.[4]

Body mass index (**BMI**)

The BMI is defined as the body mass divided by the square of the body height and is universally expressed in units of kg/m^2, resulting from mass in kilograms and height in meters.[18]

Statistical analysis

The statistical evaluation of the results, including the mean, standard deviation (SD), and standard error of the mean (SE), were calculated using the computer Microsoft office 2007. Student's t test of two independent samples has been used to compare between two means. Pearson correlation coefficient was calculated to assess the strength of correlation between two numerical variables. A P value of less than 0.05 was considered statistically significant.[19]

Results

Serum ceruloplasmin in type 2 diabetes mellitus and control groups

Table 1 provides the mean serum ceruloplasmin in type 2 diabetic patients and normal groups. The mean±SE serum ceruloplasmin was 55.9± 1.26 mg/dL in the type 2 diabetic patient group. This value was significantly (P <0.001) higher than that obtained in the control group (36.65±0.71 mg/dL). Table 2 shows the mean serum zinc in type 2 diabetic patients and normal groups. The mean±SE serum zinc was 39.19± 0.46 ug/dL in the type 2 diabetic patient group. This value was significantly (P <0.001) lower than that obtained in the control group (65.27± 2.5 ug/dL). Table 3 shows a significant positive correlation between serum ceruloplasmin with the age of diabetic patients (P <0.001), while a significant negative correlation between serum ceruloplasmin with serum zinc of diabetic patients (P <0.001).

Table 1: The mean ± SE of serum ceruloplasmin in normal and type 2 diabetes groups.

Groups	Ceruloplasmin (Mean±SEM)	P value
Type 2 diabetes mellitus	55.9 ± 1.26 m/dL	<0.001
Control	36.65± 0.71 mg/dL	

Table 2: The mean ± SE of serum zinc in normal and type 2 diabetes mellitus groups.

Groups	Zinc (Mean±SEM)	P value
Type 2 diabetes mellitus	39.19± 0.46 ug/dL	<0.001
Control	65.27± 2.5 ug/dL	

Table 3: The correlation between serum ceruloplasmin with various parameters in type 2 diabetes mellitus.

No.	parameters	Pearson's correlation (r)	P value
1-	Ceruloplasmin - age	0.383	P <0.001
2-	Ceruloplasmin - zinc	-0.354	P <0.001
3-	Ceruloplasmin - BMI	0.009	P = 0.928

Discussion

Diabetes mellitus is considered a metabolic disorder that affects carbohydrates, lipids, proteins, vitamins, minerals, and water metabolism. The purpose of this study was to determine serum ceruloplasmin (Cu-transporter plasma protein) and serum zinc as essential micromineral to investigate the effect of the disease upon micromineral metabolism. In recent years, chronic diseases, such as diabetes and hypertension, have been shown to be major causes of death worldwide.[20] The prevalence of diabetes in developed countries has reached immense proportions, which represent a major public health problem. In the current study, the number of females was found to be more than males in both groups, i.e., in diabetics with complications and diabetics without complications. This finding was consistent with the statement that type 2 diabetes is more common in women.[21] In the present study, serum ceruloplasmin levels were significantly high in diabetic subjects compared to healthy controls (P <0.001). Similar findings have been found in various other studies.[22,] The raised ceruloplasmin levels found in diabetic subjects in the present study are due to a compensatory mechanism. By keeping Fe in Fe^{3+} state, ceruloplasmin prevents it from undergoing the redox cycles necessary to initiate toxic effects. Ceruloplasmin converts Fe^{2+} into Fe^{3+} and inhibits Fenton reaction because Fe^{+2} when oxidize to Fe^{+3}. The free electron causes hydrolysis H2O2 to OH- and OH that attack components of the cell and ultimately cause damage to the cell.[23] Many trace elements are important for human metabolic function. Numerous studies have demonstrated the essential roles of trace elements as chromium, zinc, magnesium, selenium, vanadium, molybdenum, and manganese in insulin action and carbohydrate metabolism. The actual role of these trace elements in the pathogenesis and progress of diabetes is still unclear.[24] The observed alterations in the status of these elements in diabetics have been attributed to hyperglycemia and increased protein glycosylation seen in this condition. In this study, zinc levels in diabetics were lower than in the control group. This finding was in agreement with the other results,[25] but this finding was contradictory with the findings of the research.[26] In the mammalian pancreas, zinc is essential for the correct processing, storage, secretion, and action of insulin in beta (β)-cells. Insulin is stored inside secretory vesicles or granules, where two zinc (Zn^{++}) ions coordinate six insulin monomers to form the hexameric-structure on which maturated insulin crystals are based.[27] It is also known that, like most other chronic disorders, diabetes increases the excretion of minerals. Hyperglycemia in diabetes is usually associated with hyperzincuria and increased urinary loss of Zn^{++}, which is responsible for decreases in total body Zn^{++}.[28] Zinc has antioxidant properties; thus, it can stabilize macromolecules against radical induced oxidation. Zinc is a component of the important antioxidant enzyme superoxide dismutase (Cu-ZnSOD). Thus, the protection of this antioxidant against free radicals generated in the disease will be diminished.[29] It is also very important to note that zinc concentration regulates the metabolism of other very important members of the antioxidant defensive system. Vitamin A (an antioxidant) is dependent on adequate zinc level for its release from the storage site in the liver and metabolism.[29]

Conclusion

The mean serum ceruloplasmin in type 2 diabetic patients was significantly higher than that of healthy individuals. So measurement of serum ceruloplasmin may be used as a complementary biomarker in the diagnosis of diabetic patients. The mean serum zinc in type 2 diabetic patients was significantly lower than that of healthy individuals. So diabetes decreasing essential trace elements that have an important role in metabolism. Based on

current and experimental studies, it is concluded that elevated serum ceruloplasmin and lowered zinc within its laboratory normal range might be an early and sensitive marker for oxidative stress. It is recommended to determine other microelements in type 2 diabetes mellitus and study of gene expression of ceruloplasmin.

Competing interests

The authors declare no competing interests.

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