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Correlation and Comparison of Some Trace Elements and Their Related Proteins in Type 2 Diabetes Mellitus in Kalars City/ Kurdistan-Iraq

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Abstract

The serum levels of trace elements alterations have been linked with Type 2 Diabetes mellitus (T2 DM) and its complication. The Objectives of this study is to detect the correlation relation between serum Chromium (Cr), Magnesium (Mg), Zinc (Zn), Copper (Cu), and Iron (Fe) with Fasting Blood Sugar (FBS) in patients with type two diabetes mellitus, also to study the comparison of Ferroxidase, Transferrin, and Total iron binding capacity in patients with type two diabetes mellitus with control groups. A total of forty subjects were included in this study, they were divided in two groups (control and diabetic patients). Fasting blood samples were taken from all participants and analyzed for levels of Cr, Mg, Zn, Cu, and Fe. The concentration of serum trace elements of each sample was determined by using Inductively Coupled Plasma Optical Emission Spectroscopy. A significant negative correlation was found between Mg, Zn, and Fe level in Type-2 diabetics with FBS, whereas serum copper level shows significant positive correlation with FBS, and chromium shown no significant correlation with FBS. Ferroxidase, and Total binding capacity showed a significant increase, while a significant decrease was found in Transferrin of T2DM

Keywords: Type2 diabetes mellitus, Trace elements, Ferroxidase, Transferrin, Total Iron biding capacity

1. Introduction

Diabetes mellitus (DM) is an endocrine disorder which cause hyperglycemia. This occur due to either absolute or relative deficiency of insulin, cause derangement in carbohydrate, fat, and protein metabolism [1].

In type two diabetes mellitus (T2DM) disorder characterized by altered glucose metabolism and insulin secretion cause the hyperglycemia [2].

Minerals and trace elements are essential micro nutrients needed for the normal functions of our bodies, for proper physiological functions including biochemical reactions and stabilizing components of enzymes as cofactors [3]. These essential elements serve important roles in stabilization of cellular structures at certain levels, but in adequacy prosed to alternative path way and may cause illness [4]. Some essential elements have important physiological roles and direct associative with diabetes mellitus [5].

Chromium (Cr) is an essential trace metal effective in an improvement the glucose tolerance due to the reducing of insulin resistance. A study showed that Cr supplementation improve blood glucose, insulin and glycated hemoglobin levels of T2DM patients [6].

Magnesium (Mg) is a cofactor necessary for entering of glucose into the cell, also required for carbohydrate metabolism. Mg involved in the activity of insulin. It has been reported that low Mg levels decrease the resistance of the cell to the

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oxidative stress caused by diabetes. Hypomagnesemia in type two diabetes mellitus was reported [7–9].

Zinc (Zn) is a trace elements required for the potential role of insulin action, also its crucial for the production of insulin [10]. Diabetes, insulin and Zn have a complex correlation due to the Zn homeostasis responsible for the urinary loss cause decrease in total body zinc [11–13].

Copper (Cu) is the third most abundant essential elements in the body, it serve as cofactor and stabilized many metaloenzymes [1]. It has been hypothesized that Cu poses insulin-like activity in the metabolism of lipogenesis. Human researches proved that patients with DM may have altered levels of serum copper [14].

Iron (Fe) is a mineral which is essential for health and proper body function. The major functions of iron is oxygen transport and hematopoiesis in the bone marrow [15]. Iron affect glucose metabolism. The bidirectional relation between glucose hemostasis and iron metabolism is well known (i.e. adequate iron uptake could affect glucose metabolism [16].

Ferroxidase is an α-2 globulin copper containing plasma protein. It also refers as ceruloplasmin synthesized by liver cells. It also involved in iron metabolism and antioxidant defense system [17].

Transferrin is the iron binding protein in the body and its level increase with growing iron demand but serum iron is hard to detect and evaluate in separation, due to variety and consistently differences without changes in all body iron [18].

Transferrin saturation (TSAT) is the extent of transferrin bound to serum iron which is to some extent a marker of iron absorption and a mirror of iron in the milieu of iron request. It raised slightly in a non-transferrin binding iron [19].

Total iron binding capacity (TIBC) is the tendency of the protein carrying iron (transferrin) to bind with iron [20].

The aim of this study was to find the correlation relation between serum Chromium, Magnesium, Zinc, Copper, and Iron with Fasting Blood Sugar in patients with type two diabetes mellitus, also to study the comparison of Ferroxidase, Transferrin, and Total iron binding capacity in patients with type two diabetes mellitus with control groups in patients with type two diabetes mellitus.

2. Materials and methods

The study was conducted on (20) patients' women with a history of type 2 diabetes mellitus disease for more than one years aged (30–60) years without liver, thyroid, and any chronic diseases, not being pregnant or lactating; in addition to (20) healthy women as control group within the same age range. The study was approved by the Kalar General Hospital in the period between October (2020) until December (2021), and informed written consent was taken from every subject.

About (5 ml) of blood sample were taken at least 8 h overnight fast or RBS were obtained from patients and healthy individual groups, and centrifuged at 1500 rpm for 10 min to get the serum, after that kept frozen at (−30 °C) until further analysis.

2.1. Determination of trace elements

About 1 ml of the serum was transferred to a test tube, then 1 ml of the concentrated nitric acid was added, to avoid the interference. Then 0.5 ml of (30%) H₂O₂ (Hydrogen peroxide) was added. After mixing the sample by vortex, the sample was brought very slowly to the boiling water bath for 1 h to boil at 100 °C for digest protein. Then centrifuged for 15 min, and the volume was reached to 10 ml by deionized water. The solutions were analyzed against the calibration curves by (ICP-OES). The calibration curves were prepared using different concentrations of elements (0, 0.1, 0.5, 1.0, 1.5, and 2.0) ppm the serum samples were prepared as follows: 1 mL of serum was digested into with HNO₃ and H₂O₂ and then diluted into 10 mL, using deionized water as diluent So, the determined elements were multiplied by 10 as dilution factor [21],the calibration curve.
2.2. Determination of FBS level

FBS was estimated by using of (spinreact) diagnostic kit. Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H2O2) is detected by a chromogenec oxygen accepter of peroxidase (POD). The intensity of the color formed is proportional to the glucose concentration in the sample.

2.3. Determination of total binding capacity and transferrin

Serum total binding capacity and transferrin were estimated by colorimetric method using (liner) diagnostic kit [22]. LINEAR CHEMICALS, S.L.U. Joaquin Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN.

2.4. Determination of ferroxidase

Ferroxidase activity with para-phenylene diamine (PPD) as substrate was determined spectrophotometrically by one technician as described by Menden et al. [22].

2.5. Statistical analysis

Statistical analysis was used with the SPSS software Version sixteen (USA, Chicago, IL, SPSS) and
used to compare means in diabetic mellitus and control groups. A probability (the P value) was considered significant if its less than 0.001 in all statistical analyses. The Data are expressed as Means (M)± Standard Deviation (SD).

3. Results

A total of forty subjects were used in this study, and all subjects divided in two groups as described in Table 1.

Table 1 shows characteristics variables of control and Diabetics Patients. There was a non-significant difference in age and BMI in two groups.

Table 2 shows trace elements of Diabetics patients and control healthy individuals. There was a non-significant difference in Cr in two groups, and there were a significant reduction in Mg, Zn, and Fe in two groups. While a significant increase in Cu in Diabetics patients compared to control was found.

4. Discussion

In Fig. 1, A significant decrease and the non-significant correlation of Chromium with diabetes mellitus was observed in this researchers reported the improvement effects of glucose/insulin in subject with hypoglycemia, hyperglycemia, and diabetes with no detectable effect on control subjects [23].

In Fig. 2, a significant decrease and the negative correlation of Mg with FBS in Diabetics was found and this agree with a study [24]. This may be due to drug intake, dietary insufficiency or increased excretion of magnesium from the body. Or the causes may be the abnormal metabolism of Mg and could play a role in DM and further complications [24−26]. Many researchers have shown that a reduce magnesium levels is common in plasma of diabetes mellitus with type 2 [27,28].

In Fig. 3, Diabetes has been claimed to have a significant decrease and a complex negative correlation with serum Zn level by affecting Zn homeostasis and also for the increase urinary loss which decrease the total body zinc [29]. Some researchers found reduced concentration of zinc in patients with type 2 diabetic mellitus. These results may be associated with a high amount of the Zn lost in the urine. Such loss is influenced neither by an increase in its absorption by intestinal cells nor the concomitant reduction of intestinal excretion by glycemic control in diabetic patients not compensated [29].
Another study showed no correlation between serum zinc level and severity of the disease nor duration of the illness. Some researches on Cr supplementation with impaired glucose intolerance showed no clear effect [30].

A significant increase and positive correlation shown in Fig. 4 agree with a study showed an elevated levels of Copper in T2DM which also contribute to hyperglycemia and insulin resistance [31]. The increase levels in copper ion in patients with T2DM may be attributed to hyperglycaemia that may stimulate glycation and release of Cu ions and this accelerates the oxidative stress (OS), so that, the end products of Advanced Glycation are formed, that are involved in the diabetic complication pathogenesis.

The significant increase and negative correlation of Iron in Fig. 5 agree with a study showed that the incidence and risk of anemia in patients with Type 2 DM, also the higher incidence and risk in women compared to men, this may be due to poor nutrition in women than men [32]. Fe can cause severe OS and damage of some tissues. In the body, Iron homeostasis is maintained by regulators acting at the systemic and cellular levels. The ability of the body to excrete Fe is limited; therefore, amount of Fe is particularly controlled at the level of its absorption from the gastrointestinal tract [33].

The significant increase in ferroxidase in Type 2 Diabetes shown in Fig. 6. Some studies reported a decrease in ferroxidase enzyme [34]. But our results agree with a study conducted on 75 patients with T2DM. The increase in ferroxidase activity could be due to increase in the ratio of Red blood cells and oxidative stress [1].
The decrease in transferrin levels in Fig. 7 has been shown to have a negative association with type 2 Diabetes compared to control. Also, Total Iron binding capacity in Fig. 8 has been shown higher values compared to control. These results are in an agreement result from a recent study [35]. The low levels of transferrin usually associated with inflammation, malnutrition, and liver disease. Also a study reported that TIBC associated with uncontrolled diabetes mellitus increased with reduced transferrin levels, they claimed that these results are due to the glycation of transferrin in uncontrolled diabetes mellitus patients [36].

5. Conclusion
A conclusion could be drawn that the alternation in trace elements in DM in the present study could be due to nutritional status of the individuals and the severity of the disease, because some essential
Fig. 6. Comparison of serum Ferroxidase in type 2 diabetes mellitus patients with normal controls.

Fig. 7. Comparison of serum Transferrin in type 2 diabetes mellitus patients with normal controls.

Fig. 8. Comparison of serum TIBC in type 2 diabetes mellitus patients with normal controls.
elementary are involved directly in the pathogenesis and the progression of the disease. Conflicting results have been reported in other studies, so more researches needed to assess the essential elements with different categories of age severity gender and geographic places.

References


