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Estimated Blood Levels of Zinc and Copper Among Type-2 Diabetic Patients and Their Relationship to Insulin Resistance

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ABSTRACT

Disturbances in the level of trace elements might be inducing "insulin resistance (HOMA-IR)", development, and even complications of diabetes. *Objective*: A case-control study was carried out for measurements of serum zinc (Zn) and copper (Cu) concentrations in type 2 diabetic patients (DM2) and compare them with healthy subjects, as well as to estimate their association with insulin resistance. *Materials and Methods:* The experiment was carried out in Alfardous Clinic in Brack-Alshati. It involved one hundred (100) subjects, 60 diabetic subjects and 40 healthy controls. their age from 40 to 60 years. Complete blood count (CBC), glycated hemoglobin (HbA1c), fasting blood glucose (FBG), serum insulin, Cu, and Zn levels were assessed calorimetrically. Independent two-sample t-test and Pearson's correlation had been used to assess the statistical significance. *Results:* The data confirmed that DM2 patients were characterized by significantly elevated Cu, Cu /Zn ratio serum levels, HbA1c, FBG, serum insulin, HOMA-IR, and white blood cells (WBC). Additionally, a significant decline in HOMA-β, MCV, and MCH compared with the control group ($p = 0.000$). On the other hand; there were no statistical differences in the serum Zn level between the two groups. HbA1c, FBS, insulin, and HOMA-IR levels were positively correlated with serum copper, while they had no significant correlation with serum zinc. *The study found that,* serum zinc showed no association with insulin resistance in diabetic patients, while serum copper increased with this condition.

تقدير مستوى الزنك والنحاس في مرضى النوع الثاني من الداء السكري وعلاقتهما بمقاومة الانسولين

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الكلمات المفتاحية:
النوع الثاني من الداء السكري
مقاومة الانسولين.
العناصر الندرة.
مستوى الزنك.
مستوى النحاس

الملخص

تؤدي بعض الاضطرابات في مستوى العناصر النزرة إلى "مقاومة الأنسولين، وتطور مضاعفات مرض الداء السكري. الهدف: أُجريت هذه الدراسة لقياس تراكيز كل من عنصري الزنك والنحاس في مرضى الداء السكري من النوع الثاني ومقارنتها بالأشخاص الأصحاء، وارتباطها بمقاومة الأنسولين. المواد والطرق: أُجريت الدراسة في عيادة الفردوس بمنطقة براك الشاطئ، ليبيا، في الفترة ما بين فبراير إلى أبريل لسنة 2022م. شارك في الدراسة 100 شخص، كان من بينهم 60 شخص مصابين بالداء السكري من النوع الثاني، و40 شخص من الأصحاء كعينة ضابطة، تراوحت أعمارهم بين 40-60 سنة. جُمعت منهم عينات دم لتقييم تعداد الدم الكامل، الهيموجلوبين السكري، جلوكوز الدم الصائم، تركيز هرمون الأنسولين، وتقدير مستويات النحاس، والزنك لديهم. أُستخدم اختبار **t** لعينتين مختلفتين لمعرفة الفرق بين المجموعتين ومعامل ارتباط بيرسون لمعرفة العلاقة بين المتغيرات. النتائج: أوضحت النتائج أن مرضى الداء السكري لديهم ارتفاع ملحوظ في مستوى الجلوكوز، السكر التراكمي، النحاس، نسبة النحاس/الزنك، الأنسولين، مقاومة الأنسولين، وتعداد خلايا الدم البيضاء. بالإضافة إلى ذلك: وجد انخفاض كبير في HOMA-β، متوسط حجم الخلية الحمراء ومتوسط الهيموجلوبين بالخلية مقارنة بمجموعة التحكم (**p** = 0.000)، على الجانب الآخر؛ لم تُظهر النتائج أي فروق ذات دلالة إحصائية في مستوى الزنك في المصل بين المجموعتين. ارتبطت مستويات السكر الصائم، السكر التراكمي، الأنسولين ومقاومة الانسولين ارتباطًا إيجابيًا معنويًا بنحاس المصل، بينما لم يكن لها ارتباط معنوي بمستوى الزنك في المصل. خلُصت هذه الدراسة الى ان تركيز النحاس في المصل ارتفع في مرضى الداء السكري مقارنة بالأصحاء في حين لم يظهر الزنك أي فرق معنوي بين المجموعتين.

Introduction

Due to its high incidence and associated impairment and mortality, diabetes has become a severe public health concern around the world [1]. According to the International Diabetes Federation (IDF), 463 million persons between the ages of 20 and 79 had diabetes in 2019. If nothing is done, this number is anticipated to increase to 578 million in 2030 and maybe 700 million in 2045 **[2].** According to the 2009 national non-communicable disease survey in Libya, 16.4% of people had diabetes [3]. IDF predicted that Libya has a DM prevalence of about 9.7% in 2019 [3]. Hyperglycemia, which is a symptom of type 2 diabetes, is caused by the cells

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of the pancreas gradually losing their ability to secrete insulin, typically in association with increasing degrees of insulin resistance (IR) [4,5]. A decrease in sensitivity, activity, or reactivity to the metabolic effects of insulin is commonly used to describe IR [6]. It is important since it works as the best indicator of whether type 2 diabetes may develop in the future and because, if hyperglycemia is present, it serves as a treatment target [7]. Cardiovascular diseases (CVDs) and IR have a high correlation [8]. The faster progression of diabetic kidney damage may also be caused by a route of IR [9].

For all physiological processes in the body, trace elements and minerals are required [10]. By serving as cofactors or catalysts for numerous enzymes as well as sites for maintaining the structures of enzymes and proteins, it facilitates crucial biological reactions. Therefore, an imbalance in the metabolism of trace elements and in homeostasis may be a major factor in a number of disorders [11]. A high intake of processed and energy-dense foods has been linked to mechanisms causing IR, diabetes, and its complications. This is thought to be coupled with an inadequate intake of trace elements like zinc, selenium (Se), chromium (Cr), and Copper [12]. After iron, zinc is the second-most significant trace element in the body. It has a crucial role as an anti-inflammatory and antioxidant agent [14], contributes to the construction of more than 2000 transcription factors, and functions as a cofactor for more than 300 enzymes. The secretion of insulin from pancreatic islets is significantly influenced by zinc [15]. Additionally, insulin's proper synthesis, storage, and structural stability all depend on zinc [16]. Additionally, zinc is crucial for antioxidant gene expression [17].

Many underdeveloped nations struggle greatly with zinc shortage. According to a survey, approximately two billion people in the developing world lack enough zinc [18]. The only symptoms of moderate insufficiency in humans have often slowed growth and weakened [19]. Recent studies have shown that dysregulation of zinc metabolism raises the risk of diabetes and impairs the immune system, the latter of which is a contributing factor to the etiology of DM [20].

By serving as a cofactor of the superoxide dismutase enzyme, regulating glutathione metabolism, and influencing the biological functions of metalloid molecules, copper is another crucial mineral that type 2 diabetic people require for a number of functions. Superoxide dismutase (SOD), which aids in shielding cells from superoxide radicals, needs copper to function catalytically [21]. Despite being necessary, copper is extremely harmful to cells. It can facilitate the development of reactive oxygen species (ROS), which ultimately results in cell death, due to its capacity to promote the transfer of electrons and because it is extremely reactive in oxidation-reduction interactions [22]. Alterations in the regulation of copper homeostasis may play a role in the emergence of a variety of illnesses, including cancer, cardiovascular, metabolic, and neurological conditions. Oxidative stress and serum cu concentrations are tightly connected. [23]. Mitochondrial distortion is anticipated as a result of the copper shortage, especially in metabolically active cells like pancreatic and liver cells [24]. On the other hand, recent research has prompted worries about strong links between elevated levels of copper and abnormal glucose metabolism [25]. There have been a number of researches looking at the levels and status of trace elements in diabetic patients, but the results have varied [11, 26, 27]. For this reason, our study aims to measure the levels of zinc and copper in the serum of diabetic patients and compare them with healthy people, as well as to measure the role of these elements in controlling diabetes and their association with insulin resistance.

Materials and Methods

From February to April 2022, this study was carried out at the Alfardous Clinic's laboratory in Brack-AlShatti, Libya. The participants, whose ages ranged from 40 to 60 years, included 60 diabetic patients (30 females and 30 males) and 40 healthy volunteers (20 females and 20 males), who did a meeting interview to fill out a questionnaire created to fulfill the study needs of both groups. Patients with chronic kidney illness, hypertension, thyroid gland disease, and smokers were excluded from the study. In the three months prior to the trial, none of the subjects (n = 100) used any form of mineral supplement. To determine each person's body mass index (BMI), their weight and height were measured, and waist circumference was also taken.

Fasting blood samples (10 ml) have been collected from every participant by vein puncture using a 10 ml disposable syringe between 9.00 and 11:00 A.M. after 8–12 hours of fasting. The blood samples were then divided into two aliquots of 2 and 8 ml, with the first aliquot being put into EDTA tubes for HbA1c level analysis using the Nycocard READER II, and complete blood count (CBC) analysis using the Mindray BC-20s automatic hematological analyzer.

The other aliquot was dispensed into a plain tube and allowed to finish clotting for about 30 minutes. It was then centrifuged at 3000 rpm for 10 minutes to separate serum samples used for the determination of zinc and copper by colorimetric method using Photometer 4040, as well as insulin using the iFlash Immunoassay Analyzer kit and glucose using the Biolabo KENZA ONE Automatic Biochemistry Analyzer kit. The homeostatic model of assessment (HOMA) formula was used to quantify insulin resistance (HOMA-IR) and pancreatic beta-cell function (HOMA- β) [28, 29].

$$HMA - IR = \frac{\text{glucose (mg\d)} \times \text{insulin (μIU/mL)}}{405}$$

$$\text{HOMA} - \beta\% = \frac{360\text{x Insulin}}{\text{Glucose} - 63}$$

Data were analyzed statistically using the Social Sciences Statistical Package (SPSS ver. 20), and were represented as mean ± Standard Deviation (mean ± SD). An independent two samples t-test was used to measure differences between groups. Bivariate correlations were tested with normal Pearson coefficients, and P-values less than 0.05 were considered statistically significant.

Results

Table 1 shows the study groups initial characteristics; while there were variations in their BMI and WC (p-value 0.01), there were no significant differences in age between them (P-value= 0.241). According to blood parameters, RBC, platelet count, Htc%, and hemoglobin content were not substantially different between patients and controls (P-value < 0.05), whereas WBC counts were significantly higher in patients compared to the control group (p= 0.01). HbA1C, FSG, insulin, and HOMA-IR levels were significantly higher (p=0.000) in the diabetic group compared to controls, whereas HOMA- β was significantly lower (p=0.000) in the diabetic group, as shown in Table 2.

Table 1: Baseline characteristics of the study subjects

Parameters	Diabetic (n=60)	Control (n=40)	p-value
Age (years)	50.55 ± 5.261	49.30 ± 5.08	0.241
BMI (Kg/m²)	30.1 ± 4.73	27.44 ± 0.23	0.005**
WC (cm)	104.4 ± 11.2	95.6 ±11.7	0.000 **
RBC X 10^{12}/L	4.87± 0.43	4.76 ± 0.38	0.217
HBG (g/dl)	14.48 ±1.62	14.81 ±1.20	0.283
HCT%	41.11 ± 4.23	42.77±1.20	0.123
PLT X 10^9/L	261.43 ± 78.7	257.33 ± 68.5	0.789
WBC X 10^9/L	6.8 ± 1.8	5.9 ± 1.4	0.018*

*Values are expressed as Mean ± Standard Deviation, **=significant differences at (P< 0.001), * = significant differences at (P< 0.05).*

Table 2: Biochemical parameters of the study subjects

Parameters	Diabetic (n=60)	Control (n=40)	p-value
HbA1c %	8.53 ± 1.81	5.60 ± 0.43	0.000 **
FBS (mg\dl)	176.0 ± 55.21	96.50 ± 10.87	0.000**
Insulin (µU\mL)	16.96 ± 7.36	13.3 ± 4.59	0.000 **
HOMA-IR	7.47 ± 4.32	3.21 ± 1.23	0.000**
HOMA-β%	68.74 ± 49.55	151.43 ± 57.88	0.000**

*Values are shown as Mean ± Standard Deviation, **=significant differences at (P< 0.001), * = significant differences at (P< 0.05).*

As demonstrated in Figures (1) and (2), the two-sample t-test did not reveal any significant changes in blood zinc levels across groups (P > 0.05), but it did reveal a significant rise in serum copper levels in the diabetic group when compared to controls (P< 0.05).

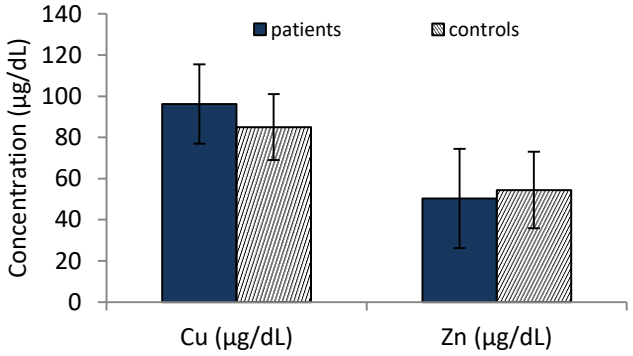


Figure 1: Comparison between diabetic patients and control group in zinc and copper concentrations

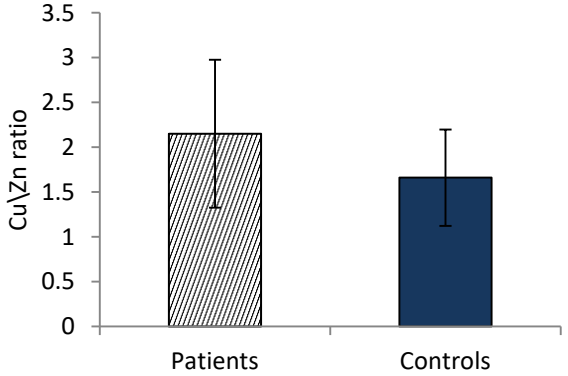
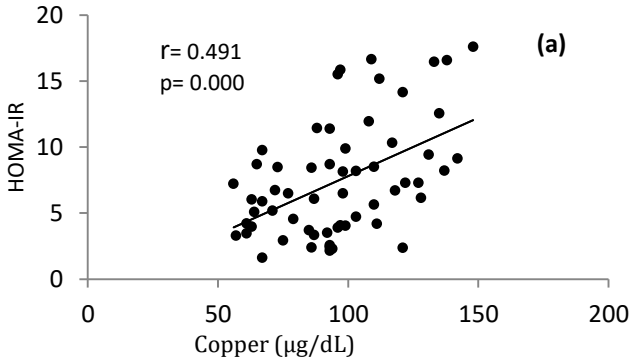


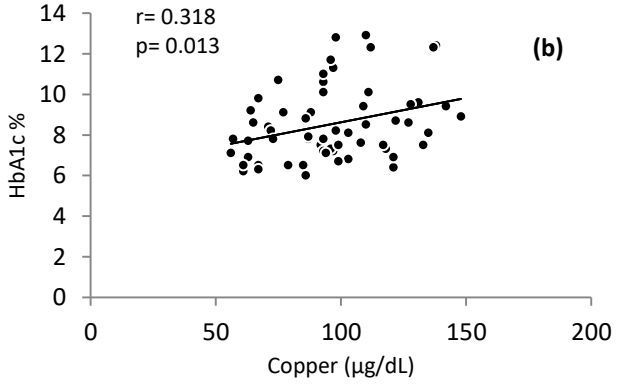
Figure 2: Comparison of Cu/Zn ratio between T2DM patients and the control group

Person correlation test were applied to demonstrate the relation between trace elements (Zn &Cu) and other parameters, strong positive correlations were found between serum Cu and FBS, HbA1c, insulin, and HOMA-IR, while no correlations were demonstrated between serum Zn and parameters as shown in figures (3) and table (3).

Table 3: Show the relation between Zn level and other parameters

Parameters	r	p-value
HbA1c %	0.015	0.912
FBS (mg\dl)	0.077	0.559)
Insulin(µU\mL)	0.245	0.059)
HOMA-IR	0.220	(0.092)
HOMA-β%	0.118	(0.369)
Cu\Zn ratio	- 0.773	(0000) **





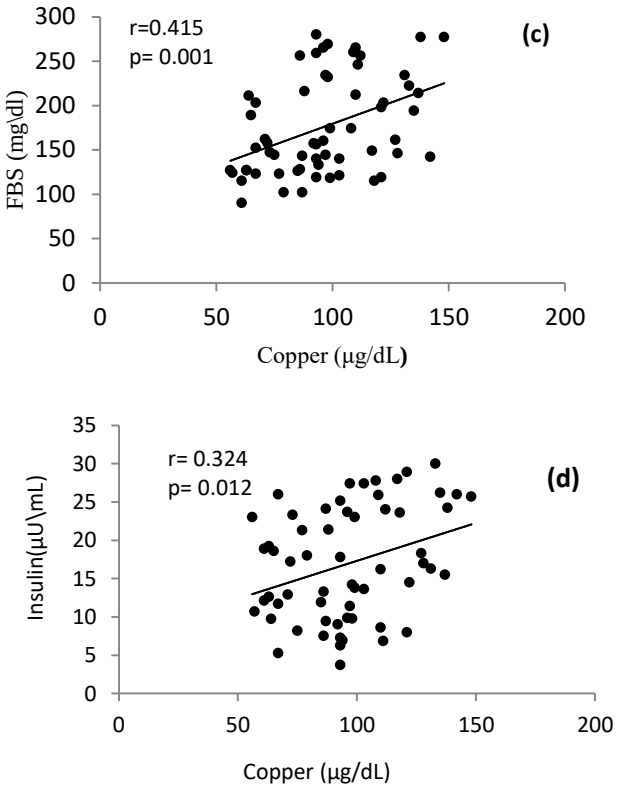


Figure 3: The relation between serum Cu and (a) HOMA-IR, (b) HbA1c, (c) FBS and (d) Insulin

Discussion

Micronutrients such as minerals and trace elements are required for the body to function normally. These components are especially useful for physiological processes [30]. Although some micronutrients are known to be implicated in the pathogenesis and evolution of diabetes mellitus, others may just be a result of depleted or altered carbohydrate intolerance and insulin resistance [31]. Diabetes mellitus can change the quantities of trace minerals. In particular, zinc and copper assessment is essential in monitoring complications due to type 2 diabetes [32]. About 50% of the world's population is at danger of zinc deficiency. Because of the binding of biogenic zinc caused by the presence of a strong chelator, a high concentration of phytate (cereal-based diets), and a low protein intake, this condition manifests in humans [33]. Zinc deficiency was reported as a risk factor for diabetes [19], due to chronic hyperglycemia and polyurea, which cause zinc loss.

Periodic serum zinc determination is mostly recommended for Type 2 diabetic patients [34]. Nevertheless, our study demonstrated no significant difference in the mean serum zinc levels between the two groups though they were lower in Diabetics. Some studies have reported results similar to our findings [35-39]. On the contrary, other investigators found high serum zinc concentrations in diabetics compared with control subjects [40-42]

This study's findings, which were in line with those of earlier research on T2DM patients [43–46], showed that the serum concentration of copper was considerably greater in T2DM patients than in control people. Hyperglycemia, which stimulates glycation and produces highly reactive oxidants that can cause tissue damage, maybe the cause of an increase in copper levels in type 2 diabetes patients [47].

According to earlier studies, the main component that contributes to the development of diabetes and may result from increased oxidative stress is insulin resistance (IR) [48]. Copper levels have a significant role in the process of oxidative enzymes (cytochrome c oxidase, superoxide dismutase, and ascorbate oxidase), which are involved in numerous biological systems [49]. However, Basaki et al. [50] demonstrated that the serum of diabetic patients had considerably low copper levels, and another study by Yakout et al. [51].on Saudi Arabian individuals revealed that , while there was no difference in the serum Zn levels between the T2DM and non-T2DM groups, T2DM had significantly lower serum copper levels than non-T2DM did. According to Mohammed et al., [52] diabetes individuals had significantly lower serum levels of zinc and copper than the control group. In addition, the gender, age, body mass index (BMI), and length of disease did not significantly affect the serum zinc and copper levels among type-2 diabetic patients. In comparison to Zinc or copper status alone, serum Cu/Zn ratio or Zn/Cu ratio may be a more accurate indicator of human metabolism [53]. A high Cu/Zn ratio has been linked to malnutrition, chronic inflammatory diseases, increased oxidative stress, inflammation, and disturbed immune function in chronic disease patients. The Cu/Zn ratio often obtains values between 0.7 and 1.0 [54]. In our study, type 2 diabetic patients had a considerably greater Cu/Zn ratio than non-diabetic controls. These findings support the research by Atari-Hajipirloo *et al*. [55].

General clinical parameters of T2DM patients, like FBS, and HbA1c, were significantly raised compared to healthy controls. Our study also demonstrated poor glycemic control in the diabetic group, with a mean HbA1c greater than 7.0 %. Poor glycemic control has been attributed to a variety of factors, including an excessively carbohydrate-rich local cuisine and a lack of exercise [56]. According to earlier research, T2DM patients' blood sugar levels were considerably higher than those of healthy controls [57, 58]. Chronic hyperglycemia in patients with diabetes predisposes them to the early development of microvascular and macrovascular complications [59]. In addition, decreasing pancreatic beta cell function and developing insulin resistance are the primary pathophysiologic defects in T2DM [60]. Many T2DM patients are unable to maintain their glycemic target with lifestyle therapy or oral hypoglycemic agents (OHA) due to the gradual nature of beta cell loss. [61]. Our study showed that the diabetic patient group had hyperinsulinemia because their mean insulin level and HOMA-IR values were higher in the patient group than in the control group. This finding also suggests a state of insulin resistance that results from receptor abnormalities and positive feedback between -cell insulin secretion and glucose levels, the main stimulus for insulin release. The obtained results matched the study conducted by Sung et al [62]. Endothelial dysfunction, atherosclerosis, and the emergence of cardiac dysfunction may all be brought on by persistent hyperinsulinemia. Insufficient control of metabolism increases these dysfunctions [63]. Insulin resistance (IR), a condition in which the body is unable to effectively respond to the insulin in the blood, is one of the potential causes of the recent rise in obesity-related mortality and morbidity worldwide [64]. The prevalence of obesity has rapidly increased across all age categories, genders, and

racial/ethnic groupings, according to recent epidemiological research, which has brought the issue to international attention [65]. Obesity may play a significant role in the development of diabetes mellitus in Libyan patients, suggesting management through diabetes patient education on the value of dietary control and increased physical activity [66]. The results of this study support previous research that links rising BMI and WC to an increased risk of T2DM. Similar to Paul et al., [67], their study found that T2DM patients had mean BMI values that were considerably higher than the matched controls (27.4 kg/m2). Additionally, Salaroli et al. [68], found that people with characteristics that were within the normal range had a lower likelihood of getting IR than those with high WC and BMI.

Several risk factors, including diabetes, hyperglycemia, hyperosmolarity, oxidative stress, inflammation, and lipid metabolic disorders, can impact RBC metabolism in diabetic individuals because they can increase aggregation, decrease cell deformability, and decrease membrane fluidity. As a result, the modification lowers the erythrocytes' rate of survival, morphology, size, and physiological capacities [69].

A frequently performed and easily accessible test is the white blood cell (WBC) count, and numerous studies have shown that this indicator of inflammation is closely connected with insulin resistance and a strong predictor of the onset of type 2 diabetes (T2D) [70, 71]. According to the current study and numerous other studies [72–74], the WBC count was statistically higher in diabetic patients than in the control group.

Hematological tests on diabetes patients in research by Al Salhen and Mahmoud [75], in El-Beida City, Libya, revealed significantly lower HCT values, hemoglobin content, RBC count, and MCV concentration than in the controls. While there were no variations in platelet counts between diabetes patients and the control group, more significant total WBC counts, MCHC, and MCH were seen in patients compared to controls.

Our investigation found no evidence of a significant relationship between zinc concentration and the glycemic indices FBS, HbA1c, serum insulin, or HOMA-IR. These results are consistent with those of Mamza et al. [76], who found no relationship between zinc concentration and FBS or HbA1c. Between the patient and control groups, Khalil et al. [77], found no discernible variation in zinc levels, and there was also no correlation with changes in glucose metabolism. Al-Hakeim et al. [78], were unable to identify any statistically significant links between T2DM and decreased zinc in their subsequent investigation. Plasma zinc was not linked with any measured elements or with metabolic markers in the Omidian et al. [79], investigation, which similarly demonstrated this.

In contrast, our research discovered a favorable relationship between Cu and glycemic indices (FBS, HbA1c, serum insulin, HOMA-IR). Similar results were obtained in numerous additional studies: in T2DM patients, copper levels were found to be positively linked with HbA1c [26, 43, 80, 81]. Knowing that copper ion is released from copper, zinc-superoxide dismutase at high glucose concentrations may help to explain, at least in part, how serum copper levels rise in diabetes conditions, even the exact mechanism is yet unknown [82]. Additionally, it was shown that Cu and HOMA- IR have a substantial positive correlation [82, 83]. Despite the fact that there was no discernible relationship between HbA1c and zinc levels in the serum, Dorreh et al. [84], discovered zinc deficiency in both IDDM and NIDDM patients. Zn and HbA1c were not shown to be significantly connected in cross-sectional research of the 3472 Chinese participants, although Cu was favorably correlated with HbA1c [85]. In type 2 diabetic patients. Eva et al. [86], demonstrated that there was a strong negative association between the mean serum zinc level and the FSG and HbA1C levels. According to a study by Al ghazeer et al., [87] male Libyan type 2 diabetic patients had significantly lower zinc and higher copper levels than the control group. Copper was positively associated with FBS and HbA1C whereas Zinc was not while Copper was strongly associated with both.

Conclusion

According to the study's findings, there were no significant differences in the serum zinc levels of diabetic patients and controls, but there was a substantial rise in the serum copper levels of diabetic patients when compared to the control group. While the blood zinc levels were not connected to any of the glycemic markers examined in this study, the serum copper levels did demonstrate a positive link with all of them.

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