



**International Journal of Biology, Pharmacy  
and Allied Sciences (IJBPAS)**

*'A Bridge Between Laboratory and Reader'*

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**INVESTIGATION OF CHANGES IN LEVELS OF SERUM ELEMENTS, LIPID  
PROFILE AND ADVANCED GLYCATION END PRODUCT IN PATIENTS WITH  
TYPE 2 DIABETES**

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**ABSTRACT**

**Background/Aims:** Diabetes is a chronic disease that is associated with impaired metabolism of trace element and lipoprotein abnormalities. The aim of this study is investigate the correlation between levels of serum elements including magnesium, copper and zinc with changes of lipid profile and total AGEs in patients with type 2 diabetes and normal men in the cities of Nowshahr and Chaloos.

**Methods:** This case-controlled study was performed on 60 patients (30 men with type 2 diabetes and 30 healthy men). In this study, lipid profile levels were determined spectrophotometrically. AGEs were estimated fluorimetrically (350 / 440 nm), serum levels of magnesium, copper and zinc were measured by atomic absorption spectrometry. The results were analysed using the SPSS software version 21 and the data were reported as Mean  $\pm$  SD.

**Results:** In this study, serum magnesium ( $P < 0.05$ ) and high-density lipoprotein and zinc ( $P < 0.01$ ) levels in the diabetic group were significantly lower than in the control group. Serum

low-density lipoprotein ( $P < 0.001$ ), copper, total cholesterol, triglycerides and AGEs ( $P < 0.01$ ) levels in the diabetic group were significantly higher than in the normal group.

**Conclusions:** Collectively, our results suggest that AGEs, magnesium, copper, zinc and Lipid Profile level assay, may be at least one cause of vascular complications of diabetes mellitus.

**Key words:** Type 2 Diabetes Mellitus, Advanced Glycation End Product, Lipid Profile, Serum Elements

## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is typically a progressive disease. Pancreatic  $\beta$ -cell dysfunction may contribute to its progression; however, the natural history of  $\beta$ -cell function in T2DM remains poorly understood. Plasma C-peptide levels have been used as an indirect measure of insulin secretory reserve [1]. T2DM is related to a cluster of interrelated plasma lipid and lipoprotein disorders [2]. These disorders occur in many patients and are characteristic of insulin resistance syndrome, the main cause of many T2DM cases. In fact, pre-diabetic individuals often show an atherogenic pattern of risk factors that includes higher levels of total cholesterol (TC), LDL, and triglycerides and lower levels of HDL than healthy subjects [3, 4]. There is evidence that each of these dyslipidemic characteristics is related to an increased risk of cardiovascular disease, the main cause of death in patients who develop type 2 diabetes[5].

Magnesium is a cofactor in the glucose transporting mechanisms of the cell membrane and various enzymes in carbohydrate oxidation [6]. Magnesium is critical to the proper functioning of many physiologic reactions, including those that are critical to the cardiovascular system. There is growing evidence that magnesium status is important in the pathogenesis and treatment of cardiovascular diseases [7]. Magnesium deficiency is associated with decreased HDL levels and increased LDL and triglyceride levels; in other words, it increases the heart coronary disease risk in diabetic patients [8]. Studies have shown that insulin and magnesium have a complex relationship with each other. Insulin can be shifted from the extracellular space to the intracellular magnesium adjust. Low intracellular magnesium concentrations are created in both T2DM and hypertension and may lead to tyrosine kinase activation defects and increased intracellular calcium concentration,

both of which cause insulin function disorder [9].

Copper is the most abundant element in the human body and can exist in two forms, reduced ( $\text{Cu}^{1+}$ ) and oxidized ( $\text{Cu}^{2+}$ ). The redox cycling capability of Cu affects its key role in electron transfer reactions [10]. It is well known that copper is effective on oxidative stress. Copper is a potent cytotoxic element in its free form due to its redox chemistry. It readily participates in Fenton and Heiber Weiss reactions to produce reactive oxygen species. The toxic effect of metal dependent free radicals is enhanced by increasing copper levels. Moreover, increasing copper levels in patients who develop T2DM might also be attributed to hyperglycemia that stimulates glycation and causes copper ions to release from the copper binding sites of proteins. The release of copper ions into the blood further accelerates oxidative stress [11].

Zn is a component of many enzymes and has many important interrelationships with the endocrine system; it is also essential for normal growth and thyroid function as well as glucose metabolism [12]. Zn deficiency in humans is mainly caused by low Zn in the diet; the availability of Zn for intestinal absorption is decreased by increasing fiber

content. The main clinical characteristics of Zn deficiency include growth retardation, delay in skeletal maturation, testicular atrophy, and hepato-splenomegaly. Other manifestations of Zn deficiency in humans include susceptibility to infection, impaired wound healing, scaly dermatitis, and diarrhea [10]. Zinc plays a key role in the synthesis, storage, and secretion of insulin, and it accounts for the conformational integrity of insulin in its hexameric crystalline form. The addition of zinc to the insulin structure will increase insulin's ability to bind to its receptor. A decrease in zinc affects the capability of islet cells to produce and secrete insulin that is particularly liable to complicate problems of Type 2 diabetics [13]. Escobar et al. reported in 1995 that low levels of zinc in diabetes mellitus is due to loss of zinc via urine or possibly to zinc loss from cells as glucose is translocated into muscle [14]. Thus, it can be stated that low levels of zinc can affect pancreas function and play an important role in DM pathogenesis [15]. Some clinical data has shown that zinc deficiency increased cataract occurrence among people with DM[16].

Patients with T2DM have an increased incidence of atherosclerotic cardiovascular disease [17] which is attributable in part to

related risk factors, including hypertension and dyslipidemia. The latter is specified by high plasma triglyceride levels, low levels of high-density lipoprotein (HDL) cholesterol, and small, dense, low-density lipoprotein (LDL) particles [18,19].

AGEs are a complex group of compounds formed through a nonenzymatic reaction between reducing sugars and amine residues on proteins, lipids, or nucleic acids [20]. One of the most important causes of disorders secondary to diabetes, such as angiopathy, neuropathy, retinopathy, deficiency in antioxidant defense system, and lipid profile disorders, is a long-term increase in glucose. The main cause is the beginning of a chain of chemical reactions (Maillard reaction) after protein glycation which causes Schiff base formation, Amadori products, and finally, advanced glycation end products (AGEs). AGEs are nonfunctional protein aggregates that can change other proteins' structure and function and are effective signal transduction pathways [21].

The pathologic effects of the process of nonenzymatic glycation are reflected in degenerative changes during aging, chronic complications of diabetes mellitus, renal failure, and neurologic diseases [22]. Previous studies have indicated that a reduction in

protein glycation and an increase in protein stability can significantly decrease the risk of complications from diabetes [21].

The objective of this study was to determine the serum levels of magnesium, copper, zinc, lipid profile, and serum TC, TG, HDL, and LDL in diabetic patients and control subjects and their status and AGEs of diabetes.

### **METHODOLOGY**

This study was performed on 60 individuals with ages of up to 35 years, including 30 men with type 2 diabetes and 30 normal men. Patients with type 2 diabetes and were selected from the Taleghani's Hospital, Chaloos, Iran, between January 2014 and April 2014 by reviewing their medical records. In addition, normal individuals were selected among academic staff of Chaloos Azad University (Iran), with normal oral glucose tolerance test and no associated disease. Criteria for the study include patients that had been diagnosed with type 2 diabetes at least five years previously, and were living in Chaloos. Exclusion criteria included diseases of liver, kidney, thyroid, parathyroid, zinc, magnesium and copper supplements, hormone therapy drugs lower Lipid Profile and smoking. 10cc of vein blood were taken from each participant in the study at the beginning of the test period. Blood samples

were collected from the cubital vein and centrifuged at 1500xg at 4°C for 10 minutes. The samples were stored at -20°C for analyses.

#### **AGEs assay**

Determination of Total AGEs done based on the spectrofluorimetric detection according to the method described by Kalousva et al (2002) [23]. The blood serum was diluted 1:50 with PBS pH 7.4 and fluorescence intensity was recorded at the emission maximum (~440 nm) upon excitation at 350 nm (Spectrofluorimeter Shimadzu RF-5000, Japan). Fluorescence intensity was expressed in arbitrary units (AU) and in AU/g protein.

#### **Lipid Profile assay**

Lipid Profile values were measured by Biosystems S.A kit (Biosystems S.A, Spain) and by the spectrophotometer device (UV-2100, American). Concentration was reported in milligrams per decilitre (mg/dl).

#### **Elements measurement:**

For magnesium after 1:50 dilution with distilled water, 1% sodium EDTA, 0.5% lanthanum in hydrochloric acid must be added to suppress phosphate interference in the air-acetylene flame and for copper and zinc undiluted vitreous was centrifuged at 3000rpm for 30 minutes and the available volume was made up uniformly to 1ml with

Mill Q water for technical reasons. This was digested with 3 ml of concentrated nitric acid perchloric acid mixture (5:1) and heated until all organic material was lost. The residue was made up to 2 ml with 5 parts distilled water. A drop of concentrated nitric acid was added to prevent adsorption of minerals on the wall of the container. The solutions were analyzed for magnesium and iron by the atomic absorption spectrophotometer (Model Varian Spectra AA 240, USA) as described earlier [24]. The instrument was calibrated with standards from Sigma Chemical Company, (St Louis, USA). Results were expressed as mg/ml of vitreous.

#### **Statistical calculations**

The statistical analysis was performed using the SPSS version 21.0 (IBM Co., Armonk, NY, USA). The significance of differences between groups was assessed using Student t-test. Pearson correlation coefficients were used to identify linear correlations between continuous variables. Data are expressed as means  $\pm$  SD ( $\pm$  Standard Deviation). Statistical significance was set at  $p < 0.05$ .

#### **RESULTS**

Table 1 shows the results of the AGEs, magnesium, copper, zinc and lipid profile in diabetic and normal subjects. As considered AGEs, copper, TC, triglycerides ( $P < 0.01$ ) and

LDL (P<0.001) concentration in diabetic samples were significantly more than normal samples, whereas magnesium (P<0.05), zinc and HDL (P<0.01) concentration in the diabetic group was significantly lower than the normal group. Also charts show the correlation between AGEs with magnesium, copper, zinc and lipid profile in the diabetic groups. As considered, AGEs

levels have no significant negative correlation with zinc (Figure 1), magnesium (Figure 2), copper (Figure 3), triglycerides (Figure 4), HDL (Figure 5) and a significantly negative correlation with LDL levels (Figure 6) (P<0.01). AGEs levels also have a significantly positive correlations with cholesterol (Figure 7) (P<0.01) in the diabetic group.

**Table 1: Levels of AGEs, Magnesium, Copper, Zinc and Lipid Profile of diabetic and control groups**

Parameter	Diabetic (N=30)	Control (N=30)	P value
AGEs (%)	253±55.99	218±42.43	<0. 01*
Magnesium (µg/dl)	2.16±0.32	2.28±0.24	<0. 05*
Zinc (µg/dl)	94.1±14.33	109.33±18.94	<0. 01*
Copper (µg/dl)	107.86±12.70	95.93±16.50	<0. 01*
TC (mg/dl)	181±53	138±32	<0. 01*
Triglycerides (mg/dl)	160±33	139±32	<0. 01*
LDL (mg/dl)	127±19	99±15	<0. 001*
HDL (mg/dl)	41±11	48±10	<0. 01*

Results are expressed as mean ± standard deviation. \* P value is statistically significant. TC: Total cholesterol, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, AGEs: Advanced glycation end products.

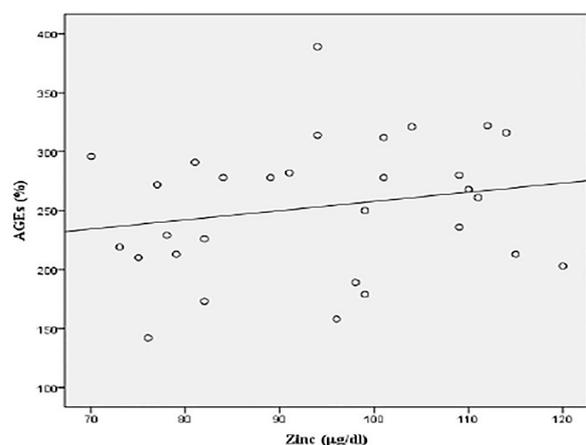


Figure 1: Correlation between AGEs and Zinc

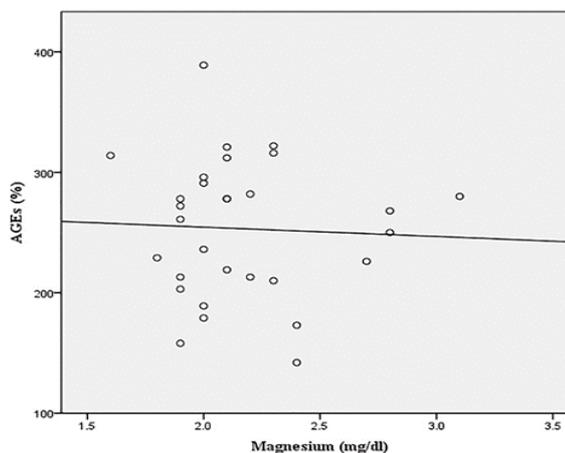


Figure 2: Correlation between AGEs and Magnesium

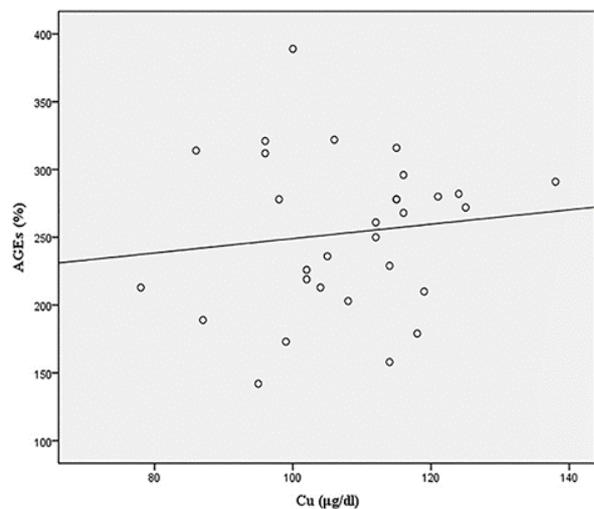


Figure 3: Correlation between AGEs and Copper

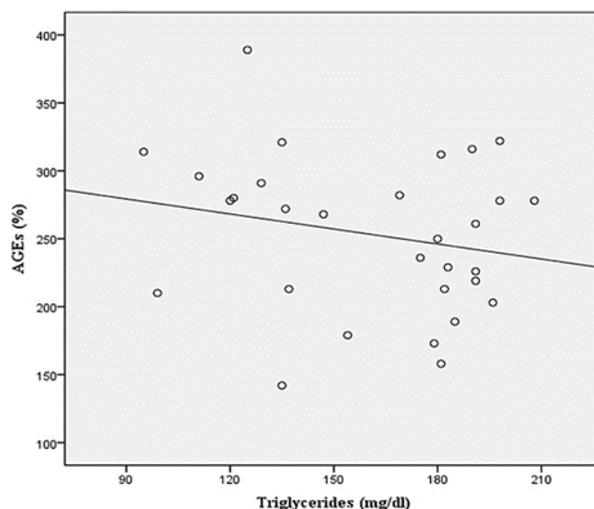


Figure 4: Correlation between AGEs and Triglycerides

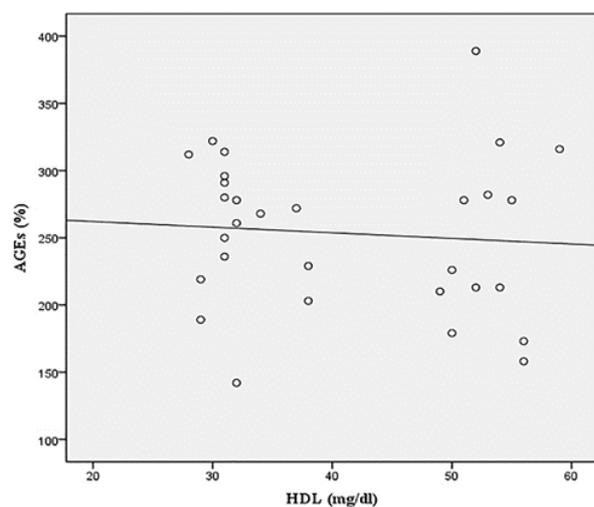


Figure 5: Correlation between AGEs and HDL

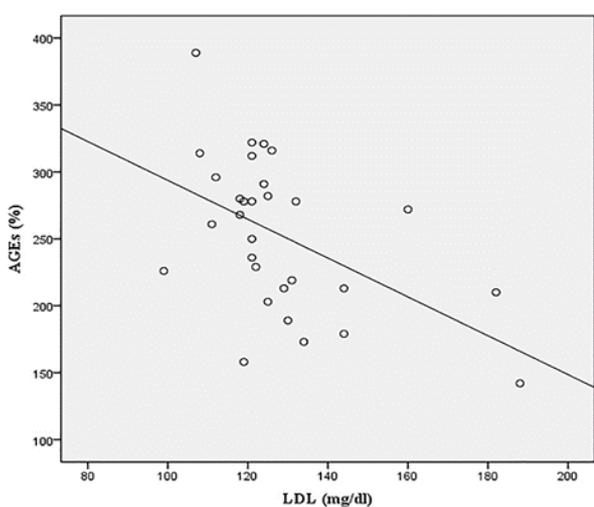


Figure 6: Correlation between AGEs and LDL

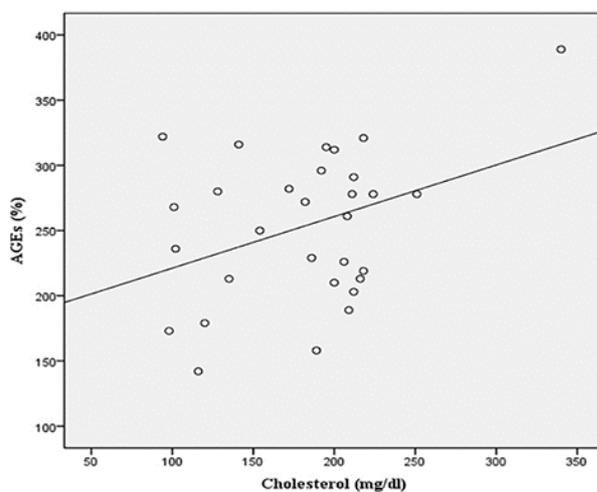


Figure 7: Correlation between AGEs and Cholesterol

## DISCUSSION

Diabetes mellitus (DM) is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. Long-term vascular complications represent a major cause of morbidity and mortality in DM patients. Moreover, different biochemical disorders related to vascular complications, such as hyperlipidemia and oxidative stress which frequently co-exist with DM [25], appear insufficient to explain the increased risk of vascular diseases. Observations suggest that additional factors may be involved in the acceleration of diabetic complications [26].

Changes of several trace elements in plasma concentrations are suspected in diabetic patients and may be involved in some metabolic disorders associated with DM. Interconnecting systems of antioxidant micronutrients (minerals) and enzymes also accomplish the body's defense against oxidative stress. Diabetes mellitus is frequently related to pancreatic enzyme disorders and oxidative stress. The present study purposed to show the role of trace element status, including copper, magnesium, zinc levels, AGEs formation, and lipid

profiles in the pathogenesis and progression of two types of DM. Moreover, it examined the relationship between these parameters and complications in type 2 diabetic patients [26]. This study also aimed to determine the correlation between AGEs and magnesium, copper, zinc, and lipid profiles in type 2 diabetics. No significant correlation between AGEs and magnesium, copper, zinc, triglycerides, or HDL were found; however, a significant negative correlation was observed between AGEs and LDL levels ( $P < 0.01$ ). The levels of AGEs, copper, TC, triglycerides ( $P < 0.01$ ), and LDL ( $P < 0.001$ ) were significantly higher in diabetic subjects. In addition, magnesium ( $P < 0.05$ ), zinc, and HDL ( $P < 0.01$ ) levels were significantly decreased. In the current study, TC, triglycerides, and LDL amounts were higher and HDL amounts were lower in the diabetic group than in the normal group. These results are in accordance with a 2004 study by Krauss et al. [5]. This similarity was also considered in a 2008 study by Gordon et al [27].

Hypertriglyceridemia, hypercholesterolemia, and AGEs were related to the oxidative modification of LDL, and therefore lead to an excess production of lipid peroxidation products and vascular complications [26]. In DM, carbohydrate disorders, lipids and the

metabolism of proteins play a predominant role in diabetic complications. Hypercholesterolemia (CHOL) and hypertriglyceridemia (TG) are mostly observed and largely related to the degree of diabetic control [7]. Serum HDLc was reported to be low in diabetic patients who develop T2DM [8]. Hyperglycemia may change lipoproteins to a form that promotes atherogenesis. LDLc levels are frequently changed in diabetic patients. Lipid peroxidation products that increase in clinical and experimental diabetes are important results of oxygen-derived free radicals stress. These products may be important in the pathogenesis of vascular complications in DM [28]. The disturbance in lipid metabolism [29] and hyperlipidemia subsequently leads to atherosclerotic diseases and cardiovascular complications. The beneficial effects of compounds decreasing elevated serum cholesterol levels in preventing coronary heart disease have been well established[21].

In the current study, amounts of AGEs were significantly higher in the diabetic group than in the normal group ( $P < 0.01$ ). These results are in accordance with a 1991 study by Makita et al. [30]. The most important cause of diabetic complications is increased serum glucose levels, which results in more protein

glycation, protein conformational change, and malfunction. Therefore, several attempts have been made to pharmacologically prevent or slow down the glycation of proteins in order to control a disease pathogenesis [21]. The increased risk of coronary artery disease in subjects who develop DM can be partially explained by lipoprotein disorders related to DM. Hyper-triglyceridemia and low levels of high-density lipoproteins are the most common lipid disorders associated with DM [31].

AGEs are probably linked to atherosclerosis in multiple ways, including enhancing endothelial dysfunction, elevating vascular low-density lipoprotein (LDL) levels by decreasing LDL uptake, promoting plaque destabilization by affecting matrix metalloproteinases, inducing neointimal proliferation, and inhibiting vascular repair in response to injury. AGEs serum levels are increased in patients with type 2 diabetes and coronary heart disease [32]. Moreover, AGEs have been localized to atherosclerotic lesions, fatty streaks, lipid-containing smooth muscle cells, and macrophages in individuals who develop diabetes. A relationship between tissue AGE concentrations and the severity of atherosclerotic lesions has also been demonstrated. There are multiple potential

mechanisms whereby AGEs may enhance atherosclerosis [20]. AGEs quench nitric oxide and impair LDL removal by trapping LDL in subendothelium and decreasing LDL receptor recognition of AGE-modified LDL. AGE's binding to apolipoprotein (apo) B impaired its hepatic clearance, induced increased retention of LDL in the aortic wall, and increased recognition by macrophages in this site. Consequently, there is increased localization of AGE-LDL in vessels and increased production of foam cells, accelerating atheroma formation[33].

Results of the present study show a significant elevation in AGEs in patients with T2DM that may be due to the following reasons: Increased oxidative stress in diabetic patients may accelerate the formation of AGEs [23]. Elevations in copper ions in the sera of diabetic patients may accelerate oxidative stress, which in turn, may enhance AGEs formation that is the actual case. This is consistent with the finding that glucose autoxidation is a transition metal-catalyzed process that produces oxygen free radicals and ketoaldehydes [34]. This argument is strengthened by the finding that the incubation of collagen with a high concentration of glucose in the presence of copper increases the rate of AGEs

accumulation, but collagen incubation with copper ions alone did not show any increase in AGEs [35].

Metal ions play an essential role in living systems, both in growth and in metabolism. Impaired metabolism of trace elements is observed in diabetic patients. It has been reported that urinary excretion of calcium, zinc, and magnesium is increased in two types of DM that causes a decrease in the levels of these elements in the blood in these patients [36, 37]. Another study reported that the levels of zinc and magnesium were significantly lower while the level of copper was significantly higher in serum of patients with T<sub>2</sub>DM[38].

The relationship between trace metals and cardiovascular complications may arise because of either a direct effect on the vascular system or indirectly through lipoprotein metabolism and oxidation. Lipid protein peroxidation can be inhibited by serum and enzymatic antioxidants the activities of which depend on the trace metal supply. Any disturbance in trace metal metabolism may cause serious consequences [10]. Many trace elements are important for human metabolic function. Numerous studies have demonstrated the essential role of such trace elements as iron, zinc, magnesium,

copper, selenium, vanadium, molybdenum, and manganese in insulin action and carbohydrate metabolism [39]. The observed changes in the status of these elements in diabetics have been attributed to the hyperglycemia and increased protein glycosylation seen in this condition[40].

Direct association of trace elements with health and disease is already established. DM, one of the most common diseases of mankind, is linked with an alteration in mineral metabolism. The data shown in Table I indicates that the serum magnesium level was significantly lower in DM patients as compared with healthy individuals ( $P<0.05$ ). The lower concentration of magnesium in diabetic patients found in the current research was consistent with the study by Diwan et al. in 2006 [41]. Similar results were also observed in the studies of De-Valk in 1992 [42]. Magnesium can play the role of a second messenger for insulin action by direct action on the entrance of glucose into the cell or by action on certain enzyme sites [43].

Magnesium deficiency is associated with many inflammatory and insulin-resistant conditions as well as diabetes mellitus. Magnesium is especially important for glucose metabolism. It is involved in over 300 different chemical processes in the human

body. For several decades, scientists have searched for a hidden relationship between DM, heart disease, high blood pressure, high cholesterol, and high blood clotting factors (high fibrinogen). Many researchers now believe that magnesium plays a central role in uniting most of these related insulin resistant diseases [44]. The term “insulin resistance” usually connotes resistance to the effects of insulin on glucose uptake, metabolism, or storage. Insulin resistance in obesity and T2DM is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output [45]. When insulin resistance occurs, glucose is produced in the blood instead of being absorbed by the cells, and that leads to type 2 diabetes [46]. One major cause of diabetic complications is serum Glucose levels increase, resulting in more protein glycation, protein conformational change, and malfunction. Therefore, several attempts have been made to pharmacologically prevent or slow down the glycation of proteins in order to control the disease pathogenesis [21]. The increased risk of coronary artery disease in subjects with DM can be partially explained by lipoprotein disorders related to DM. Hyper-triglyceridemia and low levels of high-

density lipoproteins could be the most common lipid disorders associated with DM[31].

Copper is one of these trace elements, and it plays a particular role in the cytochrome oxidase function at the terminal end of the mitochondrial electron transport chain. Activity deficiency in cytochrome oxidase function caused by a Cu deficit may contribute to the distortion of mitochondria, particularly in metabolically active tissues such as pancreatic acinar cells, enterocytes, and hepatocytes [12]. The data in Table I shows that the copper concentration was significantly higher ( $P<0.01$ ) in diabetic patients than in normal individuals, which suggests that these changes may affect the activity of the mentioned enzymes. The higher Cu concentrations in diabetic patients found in the current research was consistent with those found in the studies of Kazi et al. in 2008 [47]. Similar results were also observed in the studies of Zargar et al. in 1998 [14] and Walter et al. in 1991 [48]. Mooradian and Morley studied the impact of diabetes and its complications in 1987 and confirmed the relationship between diabetes and essential metals [49].

Some observed changes in diabetes were due to hyperglycemia and increased glycosylation

changes in serum proteins [50]. In the current study, levels of serum zinc in diabetic patients were significantly lower in diabetic patients compared with healthy subjects ( $P<0.01$ ). Similar observations were reported by Al-Marroof [51]. In other studies, the serum zinc levels were also lower in the diabetic group than in the control group, and the difference was statistically significant [52]. Moreover, it is reported that Zn levels in plasma, leukocytes, and erythrocytes is significantly lower in diabetic patients than in non-diabetics subjects. It is now clear that the predominant effect on Zn homeostasis of diabetes is hypozincemia; that may be the result of hyperzincuria or decreased gastrointestinal absorption of Zn, or both [53]. In addition, increased zinc excretion can have an effect in the form of decreased serum zinc levels. It was observed that the minimum level of zinc resulted in increased blood sugar levels due to the specific internal and external functioning of insulin on renal tubules [38]. A decrease in serum zinc levels in diabetic patients is associated with the increased urinary excretion of zinc and reduced gastrointestinal absorption [53].

Results of the present study showed that zinc and magnesium levels are decreased in T2DM blood. The loss of these minerals might be

attributed to impaired absorption and/or the excess excretion of these metals in urine (glycosuria) by these patients, which may induce a deficiency or marginal state of these minerals in the blood of diabetic patients [36, 38]. Increased copper ion levels in patients with DM might be attributed to hyperglycemia that may stimulate glycation and release copper ions from copper-containing enzymes. This argument is supported by Lin [54], who found the elevation of concentrations of both lenticular copper ions and protein-unconjugated copper ions, resulting in the decreased reactivity of copper-containing enzymes such as SOD and Cp in the lens of diabetic patients; that is the actual case.

## CONCLUSION

It can be concluded that hyperglycemia in DM is related to accelerated nonenzymatic glycation and oxidative stress. The impaired trace element metabolism of the present work may play a key role in the pathogenesis and progression of DM where copper levels increase and zinc decreases. Furthermore, calcium and magnesium levels may disturb antioxidants, induce pancreatic amylase secretion (that is considered as another diabetic complication in DM), and enhance lipid peroxidation. AGEs is increased in

diabetic patients. AGEs and the oxidative modification of lipoproteins, particularly LDL, may be at least a cause of DM vascular complications. Results of the current study also showed that T2DM may be associated with a deficiency in some essential trace elements. It seems nutrition is a critical element in any prevention strategy, and individuals with diabetes should consume an adequate quantity of foods rich in essential micronutrients in order to achieve their daily dietary requirements. It was also observed that there are disorders in the metabolism of several trace elements in DM that play a significant role in the pathogenesis and progression of the disease. Hypomagnesemia can lead to AGEs and disorders in carbohydrate and lipid metabolism. Along with antidiabetic therapy, magnesium, zinc, and copper supplementation can decrease complications caused by DM. Furthermore, all these parameters may contribute to the development of vascular complications in diabetic patients.

## Conflict of interest

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This study was supported by the Islamic Azad University of Chalooos Branch. We thank

department of research for cooperation in this work.

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