Original Research Article

Comparative study on selective trace elements in T2DM patients in correlations with their HbA1c level in tertiary care hospital in Karaikal district

Jaiprabu Jayaraj[1], Pandiamunian Jayabal[2*]

[1]Associate Professor, Department of Biochemistry, Vinayaka Missions Medical College, Karaikal, India
[2]Associate Professor, Department of Pharmacology, Vinayaka Missions Medical College, Karaikal, India
[*]Corresponding author email: **jpandiamunian@gmail.com**

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Abstract

Background: Diabetes mellitus is a chronic metabolic disorder that arises due to absolute or relative lack of insulin production by the beta-cells of the pancreas. Impaired secretion of this protein affects glucose metabolism, and consequently, results in hyperglycemia. Unregulated levels of blood glucose can lead to several debilitating conditions such as nephropathy, neuropathy, retinopathy, cardiovascular disease, stroke, and amputations of extremities. About 90 to 95% of the patients are affected by type 2 diabetes which is characterized primarily by insulin resistance, hyperinsulinemia, and beta-cell dysfunction. Trace elements facilitate numerous biochemical reactions including those related to insulin and glucose metabolism.

Aim of the study: To compare the trace element concentrations T2DM patients and healthy age-matched controls.

Materials and methods: The Study includes 22 types of II diabetic patients as cases and 22 normal individuals as controls. Fasting blood sugar (FBS) and Postprandial blood sugar (PPBS), in this cationic study, sodium, potassium, calcium, iron, zinc, and magnesium were measured in plasma of T2DM and compare with healthy controls. Fasting blood samples were collected into labeled centrifuge tubes, after an 8–12 hours overnight fast, from the subjects by venepuncture. The blood

samples were centrifuged at 2000 rpm for 10 min using a desktop centrifuge and the serum separated and kept in labeled sample bottles at −70°C until further analysis.

Results: The results showed that the plasma of T2DM patients contains significant ($p<0.05$) more sodium, calcium, copper, zinc, and magnesium compared to the plasma of age healthy age-matched controls. The results also showed that there was no change in potassium levels comparing control with T2DM patients. However, T2DM patients had significantly ($p<0.05$) less iron compared to age-matched healthy controls.

Conclusion: The present study provides significant evidence showing that altered metabolism of Cu, Zn, Cr, and Mg is strongly associated with the increased levels of HbA1c. These associations might represent a risk factor for the development of diabetic complications. Our findings indicate that it is necessary to take into consideration possible changes in the metabolism of these metals, mainly their associations with long-term hyperglycemia.

Key words

HbA1c, Diabetics mellitus, Trace elements, Oxidative stress.

Introduction

There is accumulating evidence that the metabolism of several trace elements is altered in DM and that these nutrients might have specific roles in the pathogenesis and progress of this disease [1]. Zinc (Zn) is an essential trace metal that is directly involved in the synthesis, storage, secretion, and conformational integrity of insulin monomers and that Zn assembles to a dimeric form for storage and secretion as crystalline insulin Lower levels of Zn may affect the ability of pancreatic islet cells responsible for the production and secretion of insulin, such as in type 2 diabetes [2]. Epidemiological studies have reported decreased plasma and intracellular Zn concentrations in conjunction with increased urinary Zn excretion in diabetic patients. In subjects with type 2 DM with low Zn intake, the risk of coronary heart disease increases by a factor of two to four times and is a major cause of mortality among diabetic patients [3]. Copper (Cu) and Zn play a pivotal role in the oxidant/antioxidant mechanism, imbalance of which leads to increased susceptibility to oxidative damage of tissues, thereby leading to the pathogenesis of DM or diabetic complications. The changes in the metabolism of Cu and Zn that occur during oxidative stress may be important in several processes where oxidative stress is implicated. Both the essentiality and toxicity of these metals in the pathogenesis of DM and diabetic complications are often reported [4]. Some investigators have reported the hypothesis that glycated proteins bind transition metals such as Cu and iron (Fe) and that such glycocholates play an important role in the etiology of peripheral vascular dysfunction and peripheral neuropathies in DM [5]. It was intensively investigated that chromium (Cr) acts as a blood–sugar modulator that could guard against glucose imbalances. Magnesium (Mg) is the fourth most abundant cation in the body and second in the intracellular environment. It takes part in more than 300 enzymatic reactions [6]. Deficiency of Mg has been associated with a variety of clinical conditions, including type 2 DM. Mg depletion has a negative impact on glucose homeostasis and insulin sensitivity in patients with type 2 diabetes, as well as on the evolution of complications such as retinopathy, thrombosis, and hypertension [7]. The cause of hypomagnesemia was attributed to osmotic renal losses from glycosuria, decreased intestinal absorption, and redistribution of Mg from the plasma into blood cells due to the effects of insulin [8].

Materials and methods

The Study included 22 types of II diabetic patients as cases and 22 normal individuals as controls. Fasting blood sugar (FBS) and

Postprandial blood sugar (PPBS), in this cationic study, sodium, potassium, calcium, iron, zinc, and magnesium were measured in plasma of T2DM and compare with healthy controls. Samples of blood were collected by venepuncture technique from both T2DM patients and healthy controls following consent. The patients were also given a questionnaire to fill in which contained several questions regarding their lifestyle habits and medical history for an epidemiological study. Measurement of various cations including sodium, potassium, calcium, magnesium, zinc, copper, iron and selenium was performed using inductively coupled plasma mass spectrometry, (ICP-MS) technique with the plasma of both T2DM and healthy controls.

Inclusion criteria: Subjects should have been type II diabetic for at least one-year duration without any complications.

Exclusion criteria: Patients on thyroid stimulating drugs, corticosteroids, lipid-lowering drugs, oral contraceptives, aspirin, sulphonamides and those diabetic patients with a history of diabetic retinopathy, nephropathy, and neuropathy were excluded.

Biochemical assay of parameters

5 ml Blood was collected in an EDTA tube by venepuncture technique and it was centrifuged at 3500 rpm for 10 min to separate plasma from the red blood cells. A volume of 0.2 ml of plasma was diluted with 9.8 ml deionized water to make 10 ml solution in each tube. A volume of 4 ml of nitric acid was added to 1 ml of the above solution in each tube and 10 µl of internal standard was added to each tube. The HNO3 was used to denature any proteins in the plasma in order to facilitate the measurement of the ions. Cations were measured using ICP-MS according to the standard protocol of the instrument. Values were expressed as parts per billion which were subsequently converted into mM/L values. All the serum samples were analyzed for fasting and postprandial blood sugar, serum copper, serum zinc, serum uric acid, and serum albumin. Serum FBS and PPBS were determined by the glucose oxidase and peroxidase (GOD-POD) method using a commercially available kit in a semi-auto analyzer. Normal values of serum fasting blood sugar: 70-110 mg/dl and Postprandial blood sugar: < 140 mg/dl. Estimation of serum copper and serum zinc was carried out by the colorimetric method using the commercial kits available in the market. Normal reference values of serum copper in males - 80 - 140 µg/dl; while in females, it is 80 - 155 µg/dl. The normal range of serum zinc levels is - 60 – 120 µg/dl.

Statistical Analysis

All the values were expressed as mean ± SD and $P < 0.05$ was considered statistically significant. Statistical significance of the differences between the mean values was analyzed by one way ANOVA test using SPSS 16 statistical analysis software. Correlations between different variables were analyzed using Pearson's correlation coefficients (r). Sample size was calculated using ($\alpha = 0.05$, $\beta = 0.2$, $\sigma = 18$, $d = 11$) formula.

Results

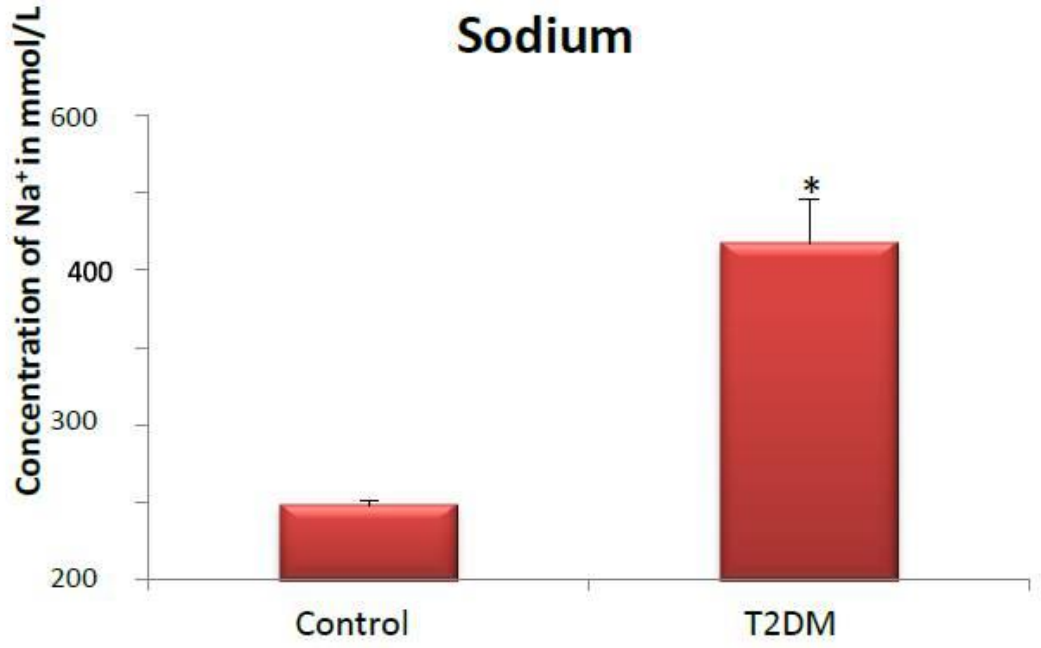
In this cationic study, sodium, potassium, calcium, iron, zinc, and magnesium were measured in plasma of T2DM and healthy controls. A total of 22 T2DM patients and 22 healthy controls were involved in this particular study. The results show that the plasma of T2DM patients contains significant ($p<0.05$) more sodium, calcium, copper, zinc, and magnesium compared to the plasma of age healthy age-matched controls.

The mean age of diabetic patients was 60.09 versus 58.92 years of non-diabetic subjects. The diabetic patients were generally heavier than the control subjects (**Table - 1**). The results of the BMI indicated that the diabetic subjects were overweight. There was a significant difference in the BMI of the diabetic patient when compared with the control group. Fasting blood glucose and HbA1c were significantly higher in diabetic patients than in non-diabetic subjects.

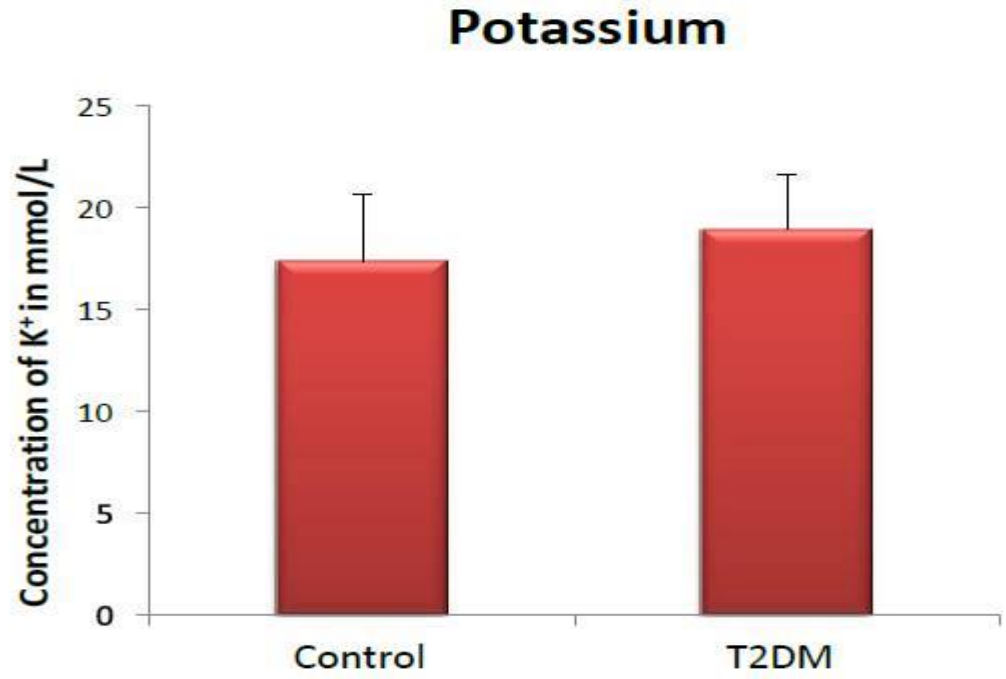
Table – 1: Demographic data of patients.

	Healthy controls (n=22)	**T2DM (n=22)**
No of subjects	22	22
Age group	30-60 years	30-60 years
Gender	14 Male and 8 Female	12 Male and 10 Female
BMI (kg/m^2)	21.37 ± 1.28	26.59 ± 1.82
Fasting blood glucose (mmol/L)	10.21 ± 3.56	4.23 ± 0.15
HbA1c (%)	9.18 ± 2.30	5.27 ± 1.28

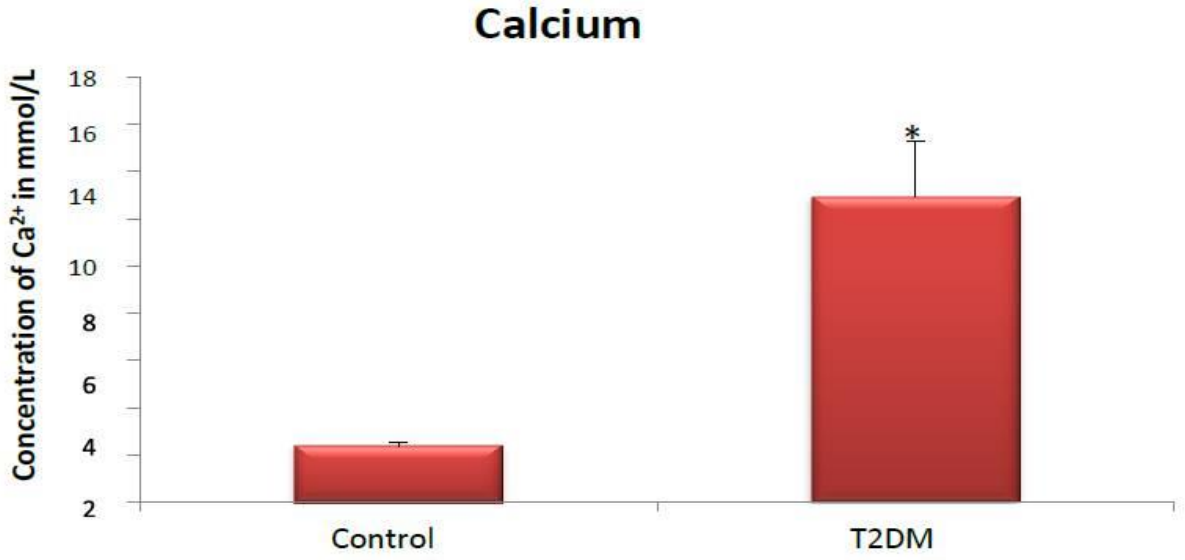
Graph – 1: Comparison of sodium level in T2DM and healthy controls.



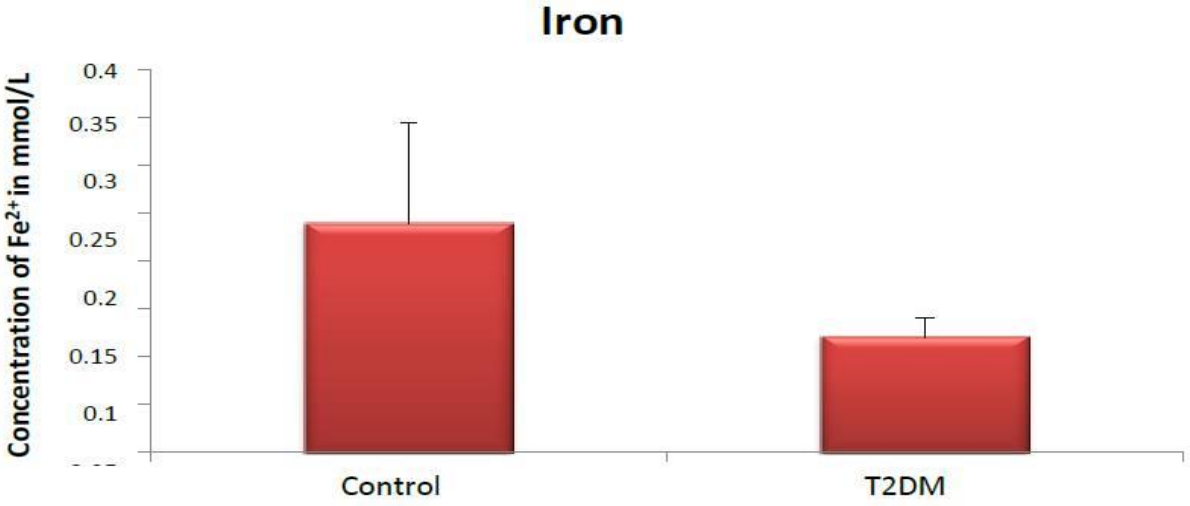
Graph – 2: Comparison of potassium level in T2DM and healthy controls.



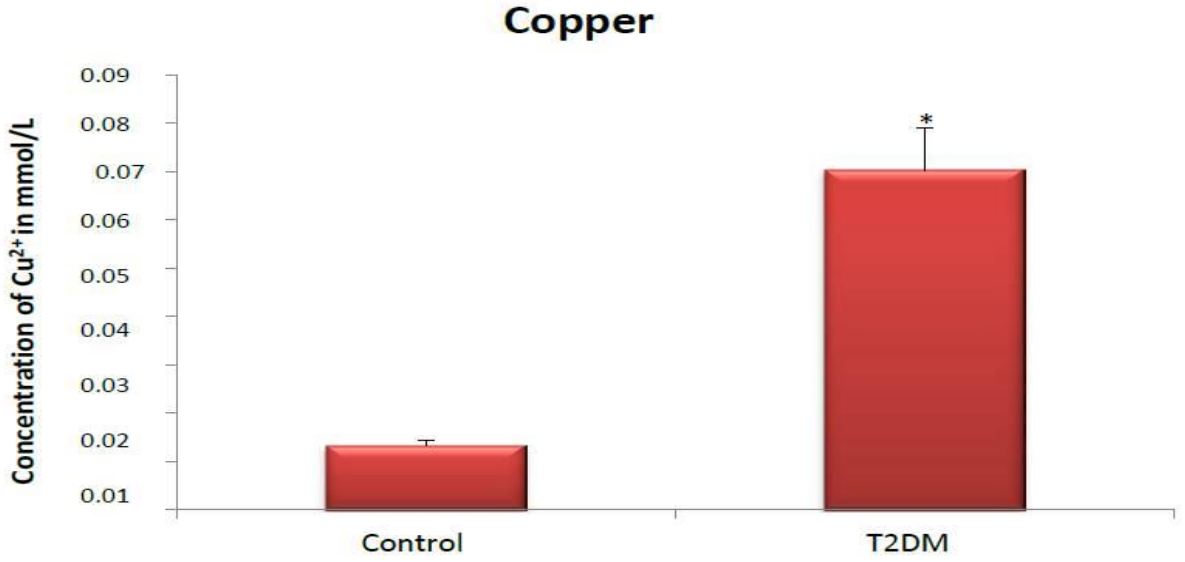
Graph – 3: Comparison of calcium level in T2DM and healthy controls.



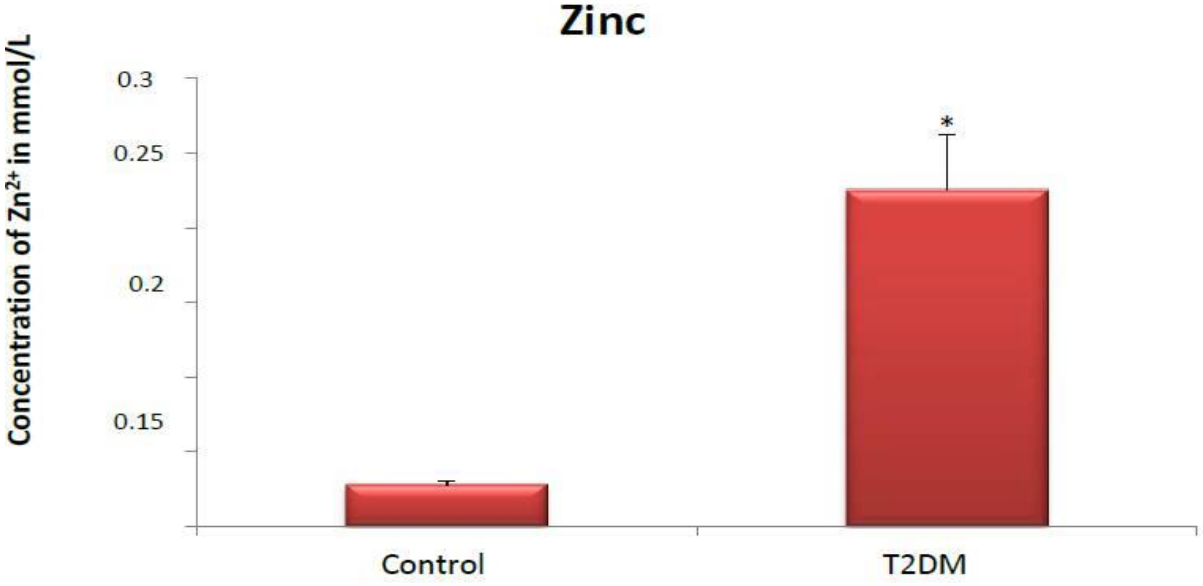
Graph – 4: Comparison of iron level in T2DM and healthy controls.



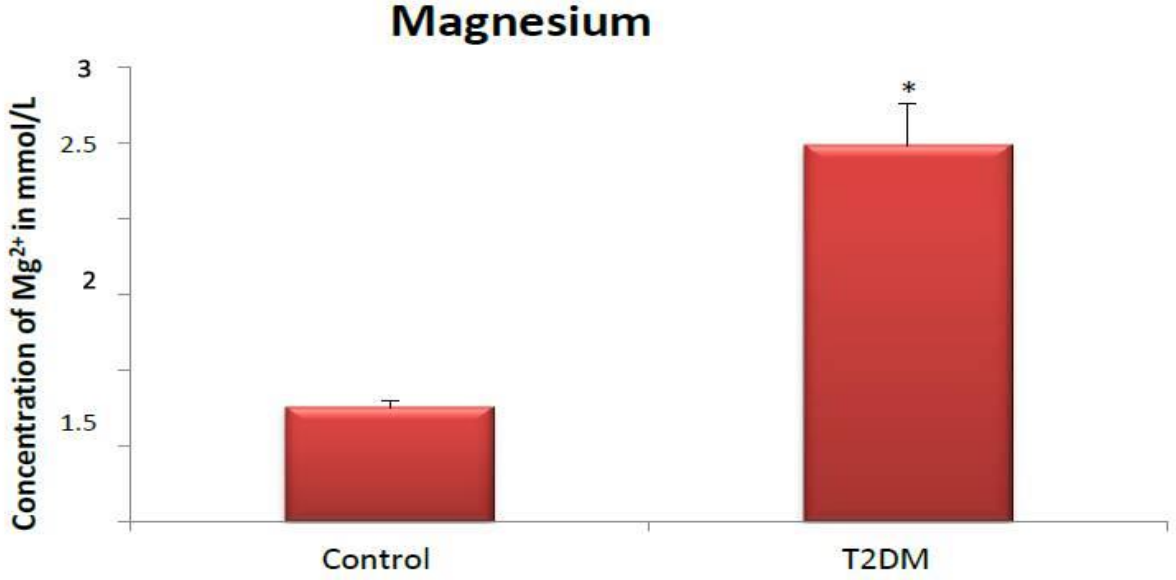
Graph – 5: Comparison of copper level in T2DM and healthy controls.



Graph – 6: Comparison of zinc level in T2DM and healthy controls.



Graph – 7: Comparison of magnesium level in T2DM and healthy controls.



Graph - 1 showing plasma sodium (Na^+) concentrations (mmol/L) in T2DM compared to controls. Values were expressed in terms of mean ± SEM, n=22, *p<0.05 for T2DM compared to control group.

Graph - 2 showing plasma potassium (K^+) concentrations (mmol/L) in T2DM compared to controls. Values are mean ± SEM, n=22. Note that there was no significant difference in K^+ levels in both control and diabetic groups.

Graph - 3 showing plasma calcium (Ca^{2+}) concentrations (mmol/L) in T2DM compared to controls. Values were mean ± SEM, n=22, *p<0.05 for T2DM compared to control group.

Graph - 4 showing iron (Fe^{2+}) concentrations (mmol/L) in T2DM compared to controls. Values were mean ± SEM, n=22, *p<0.001 for T2DM compared to control group.

Graph - 5 showing plasma copper (Cu^{2+}) concentrations (mmol/L) in T2DM compared to

controls. Values were mean ± SEM, n=22, *p<0.001 for T2DM compared to control group.

Graph - 6 showing plasma zinc (Zn^{2+}) concentrations (mmol/L) in T2DM compared to controls. Values were mean ± SEM, n=22, *p<0.001 for T2DM compared to control group.

Graph - 7 showing plasma magnesium (Mg^{2+}) concentrations (mmol/L) in T2DM compared to controls. Values were mean ± SEM, n=22, *p<0.001 for T2DM compared to control group.

Discussion

It is well understood that CVD is the leading cause of mortality and morbidity in T2DM patients as there is an increased risk of CVD in this particular population [9]. Generally, there are two types of DM namely, T1DM and T2DM. T1DM is also called insulin-dependent or early onset diabetes, which is diagnosed during childhood and accounts for about 5-10% of DM. T1DM is due to the autoimmune destruction of pancreatic β cells in islets of Langerhans resulting in a decrease of insulin production [10]. T1DM is commonly associated with microvascular complications including nephropathy and retinopathy and it also predisposes to CHD. Several studies were published during the last century evaluating the various cations and trace elements in T2DM [11]. However, significant changes were observed within similar reports and these differences were shown to be a result of various factors including age, genetic factors, dietary habits, medication and glycaemic control of patients [12]. Alterations in the levels of various cations could also result due to chronic hyperglycemia and possibly modulation of glucose homeostasis by micronutrients themselves [13]. Blood plasma is considered to be a tissue pool for various cations and single trace elements. However, recently, it became evident that the micronutrient level of plasma is a reflection of cations and trace elements only in cases of severe deficiency or in excess [14]. Several underlying factors for excess and deficiency of these various cations in plasma include infection, regulation of hormones, stress, circadian rhythm, impaired homeostatic regulation. Hyperglycaemia in patients with T2DM is a common clinical problem in which variable changes in plasma sodium (Na+) concentration can occur [15]. Acute studies suggest that hyperinsulinemia may cause sodium retention and increased sympathetic activity, which will be an important cause of hypertension [16]. The results from the Atherosclerosis Risk in Communities (ARIC) Study, suggest that serum potassium is an independent predictor of incident T2DM. Essentially, maintenance of normal potassium homeostasis is an important limiting factor in the therapy of cardiovascular disease [17]. A diet deficient in potassium intake has a critical role in regulating blood pressure in primary hypertension and may potentially increase the risk of stroke studies have shown that hypokalemia could be a possible risk factor for T2DM [18, 19]. Serum potassium levels affect insulin secretion by pancreatic β-cells. Serum potassium in randomized controlled trials of thiazide diuretics was found to be inversely associated with glucose which might possibly be a result of oral potassium supplementation [20]. Experimental evidence also suggests that thiazide-induced hypokalemia can lead to reduced insulin secretion. In insulin-responsive tissues such as skeletal muscle and adipose tissue, calcium is essential for insulin-mediated intracellular processes. A very narrow range of [Ca2+]i is needed for optimal insulin-mediated functions [21]. Changes in [Ca2+] I in primary insulin target tissues possibly contribute to peripheral insulin resistance via impaired insulin signal transduction, resulting in a decreased glucose transporter-activity [22]. It has been shown that Mg2+ deficiency is associated with serious cardiovascular diseases, such as cardiac arrhythmia and coronary heart disease, as well as with risk factors including hypertension, hypercholesterolemia and diabetes mellitus and moreover, a deficiency of serum magnesium has been reported in T2DM. Nevertheless, the effects of zinc supplement, if dietary intake is adequate, are incompletely understood. More clinical data

on diabetic patients who are at increased risk of zinc deficiency would be helpful as zinc has an insulin mimetic effect and moreover, it also protects against the oxidative damage associated with the disease [23]. Additionally, oxidative damage in diabetic patients may result in lower antioxidant micronutrient status, especially trace elementsIn subjects with insulin dependent diabetes mellitus (IDDM), zinc concentrations have been demonstrated to be lower in leucocytes and erythrocytes than in serum, while no such alteration has been found with the copper. No definite association has been explained between copper concentrations and the clinical status of patients with diabetes mellitus [24]. T2DM is a common manifestation of hemochromatosis, a disease of iron overload. Iron is a catalyst involved in the formation of hydroxyl radicals, which are powerful pro-oxidants that attack cellular membrane lipids, proteins, and nucleic acids. Formation of hydroxyl radicals catalyzed by iron contributes initially to insulin resistance and subsequently to decreased insulin secretion which leads to the development of T2DM. Thus, iron plays a major role in several steps of insulin action and glucose metabolism [25].

Conclusion

The results of the current work indicated an imbalance in the levels of trace elements in type 2 DM. We found significantly higher levels of significantly reduced the levels of Zn, copper, magnesium iron sodium and potassium Mg in patients with DM when compared with healthy subjects. Hyperglycemia induces free radicals and impairs the endogenous antioxidant defense system in diabetes mellitus. In our present study serum, copper and zinc levels were altered in type II DM.

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