Original article

# Evaluation of serum copper, magnesium and glycated haemoglobin in type 2 diabetes mellitus

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***Abstract***

***Objectives:*** Type 2 diabetes mellitus (DM) is associated with the alteration of trace elements like copper, zinc, magnesium and manganese which may be a contributing factor in the progression of DM and its complications1. Objective of the present study was to estimate serum copper, magnesium, zinc, manganese and glycated haemoglobin in patients with type 2 DM and compare it with controls (non diabetic healthy subjects).

***Materials and methods :*** This cross sectional analytic study was conducted in 200 subjects, out of which 100 were type 2 diabetes mellitus patients as cases (52 males, 48 females) and 100 were non diabetic healthy subjects as control (40 males, 60 females). Serum copper, magnesium, zinc, manganese, glycated haemoglobin were measured by using the auto analyzer Beckman Coulter DXC 600. Serum copper, magnesium, zinc, manganese were measured by modified spectrophotometric micromethod using Guanidine hydrochloride and Bathocuprine disulphonate disodium salt (BCDS).

***Results and observations :*** The mean age of type-2 diabetic patients were (48±10.44) years versus (42±9.37) years of non-diabetic control subjects. The type-2 diabetic patients were generally heavier (BMI 25.04±3.29) than the control subjects(BMI 25±2.71 ) where p<.05. The BMI, waist circumference, hip circumference and fasting blood glucose were significantly higher in the type-2 diabetic population when compared to the non-diabetic control population of the study. Type-2 diabetic patients had higher levels of fasting blood glucose, glycated hemoglobin and lipoproteins than non-diabetic control patients. The (Mean±SD) of plasma glucose in type-2 diabetic patients and controls were (7.65± .25) m.mol/l and (4.99±.95)m.mol/l respectively, (p<.001) and the mean HBA1C of type-2DM patients and controls were (8.41±1.04) mg/dl and (5.87± .98) mg/l respectively (p<.001). The (Mean±SD) of Zn, Cu and Mg for type-2 diabetic patients and controls were (.941±.246) mg/l, (.771±.483) mg /l, (14±3.613) mg/l and (1.21±.105) mg/l , (1.142 ± .239)mg/l, (18 ± 1.72)mg/l respectively where p<.001 and For Mn was (.091±.049) mg/l and (0.106±0.030) mg/l respectively (p<o.o1). The TAG, Cholesterol, LDL-C and HDL-C for type-2 diabetic patients and controls were (226±124.16)mg/l,(192±42.11)mg/l ,(113±34.52)mg/l, (37±5.49)mg/l and (138±89.23)mg/l, (165±33.06)mg/l, (95±30.05), (41±9.003)mg/l respectively and p<.001. A significant correlations were found between serum Zn and TAG of type-2 DM(r = 0.209) and HDL-C of type 2 DM(r=.199) where p<.05 and non significant relationships were found in between Zn and lipid profile (TAG, Cholesterol, LDL-C, and HDL-C) of control group. A significant correlation found between serum magnesium and TAG of control where p<.01 and non significant correlations were found in serum Mg and Total cholesterol, HDL-C, LDL-C of both type-2 diabetic and control. There were significant correlations in between serum Cu and Mn and TAG of control where p<.05 and non significant correlations were found in between Cu and Mn and other component of lipid profile of both type -2 DM and control.

***Conclusion :*** Patients with type 2 DM had altered metabolism of copper, zinc, magnesium , manganese and this may be related to the increased level of glycated haemoglobin. Impaired metabolism of these trace elements may have a contributory role in the progression of DM and its complications.

***Keywords :*** Copper, Hypomagnesemia, Glycated haemoglobin, type 2 diabetes mellitus, Oxidative Stress

## Introduction

Type-2 DM is an endocrinological disease associated with hyperglycaemia characterised by both insulin resistance and defective insulin secretion[1]. A relationship between DM and minerals is frequently reported. Alteration in the metabolism of trace elements like copper, magnesium is associated with DM[2]. Trace elements are accepted as essential for optimum health, because of their diverse metabolic characteristic and functions[3]. Trace elements participate in production of reactive oxygen species (ROS), which contribute to oxidative stress. Oxidative stress contributes to the pathogenesis of many diseases including DM. Previous studies have shown that copper causes oxidative stress[1-5]. Copper acts as a pro-oxidant and may participate in metal catalyzed formation of free radicals[2]. The increased production of free radicals is likely to be associated with development of type 2 DM. Magnesium is an essential element involved in glucose homeostasis & a cofactor for various enzymes in carbohydrate metabolism. It also involves at multiple levels in insulin secretion, binding and activity. Reduced level of magnesium has been documented in type-2 impact on glucose homeostasis and insulin sensitivity in type 2 DM patients[6]. Hypomagnesaemia may also have some effect in the development of diabetic complications with other risk factors[7].

Zinc is an essential trace metal that is directly involved in the synthesis, storage, secretion, and conformational

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integrity of insulin monomers and that Zn assembles to a dimeric form for storage and secretion as crystalline insulin8,9. Lower levels of Zn may affect the ability of pancreatic islet cells responsible for the production and secretion of insulin, in type-2 diabetes[10]. 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[11,12].

**Material and methods**

The study was approved by the Ethical Committee; a written informed consent was obtained from all participants in this study. A total of 100 patients (aged 30-70 years) with type-2 DM recruited from BIRDEM's Medicine and Endocrinology departments. The diagnosis of type-2 DM was confirmed by biochemical investigations as per WHO criteria Patients were excluded when diagnosed with type 1 DM, acute complications such as severe infection, major operations, trauma, GI disorders, severe cardiovascular/ respiratory diseases, pregnant and breast feeding women. Patients taking supplements such as antioxidants, vitamins, minerals were also excluded. Age and sex matched 100 controls were recruited after clinical and biochemical evaluation. The baseline demographic data and family history were obtained. Three ml of venous blood sample was collected for estimation of blood glucose, HbA1c, magnesium, manganese, zinc and copper. Serum magnesium was measured by a timed end point calmagite method[8] and serum copper, manganese; zinc was measured by modified spectrophotometric micro-method using guanidine hydrochloride and HbA1c concentration was measured using HPLC reference method[10]. All the above mentioned parameters were measured using the autoanalyzer Beckman Coulter DXC 600. Statistical analysis of data was performed using the SPSS (Version 15.0). For the comparison of values between the groups, students't' test was used, represented by 'p' value. Statistical significance was considered at a 'p' value of < 0.05. For the correlation, Pearson's correlation coefficient was used.

**Results**

One hundred (100) patient with type-2 DM as cases (52 males, 48 females) and 100 healthy subjects as control (40 males, 60 females) comprised the study group. The mean age of type-2 diabetic patients was (48±10.44) years versus (42±9.37) years of non-diabetic control subjects. The type-2 diabetic patients were generally heavier (BMI 25.04±3.29) than the control subjects (BMI 25±2.71) where p<.05(Table 1). Table-1 also shows The BMI, waist circumference, hip circumference and fasting blood glucose were significantly higher in the type-2 diabetic population when compared to the non-diabetic control population of the study. It has been established that type-2 diabetic patients had higher levels of fasting blood glucose, glycated hemoglobin and lipoproteins than non-diabetic control patients (table-2). Table-2 showed The (Mean ± SD) of plasma glucose in type-2 diabetic patients and controls were (7.65± .25) m.mol/l and (4.99±.95)m.mol/l respectively, (p<.001) and the mean HBA1C of type-2DM patients and controls were (8.41±1.04) mg/dl and (5.87±.98) mg/l respectively (p<.001). The (Mean±SD) of Zn, Cu and Mg for type-2 diabetic patients and controls were (.941±.246) mg/l, (.771± .483) mg/l,(14±3.613)mg/l and (1.21±.105) mg/l, (1.142 ± .239)mg/l, (18 ± 1.72) mg/l respectively p<.001 and For Mn were (.091±.049) mg/l and (0.106±0.030) mg/l respectively (p<o.o1). The TAG, Cholesterol, LDL-C and HDL-C for type-2 diabetic patients and controls (226±124.16) mg/l, (192±42.11) mg/l, (113±34.52)mg/l, (37 ±5.49) mg/l and (138±89.23) mg/l, (165±33.06) mg/l, (95± 30.05), (41±9.003) mg/l respectively and p<.001. A significant correlations were found between serum Zn and TAG of type-2 DM (r = 0.209) and HDL-C of type 2 DM(r=.199) where p<.05 and non significant relationships were found in between zn and lipid profile ( TAG, Cholesterol, HDL-C, and HDL-C) of control group (table-3,4). There was a significant correlation was found between serum magnesium and TAG of control where p<.01 and non significant correlations were found in serum Mg and Total cholesterol, HDL-C, LDL-C of both type-2 diabetic and control (table-3). Significant correlations found in between serum Cu and Mn and TAG of control where p<.05 and non significant correlations were found in between Cu and Mn and other component of lipid profile of both type -2 DM and control ( table 3, 4).

**Table1 :** Descriptive physical characteristics of diabetic patients and controls

| Characteristics | Type-2 DM (case) | Non-Diabetic Controls | P Value |
|---|---|---|---|
| Age in years | 48±10.44 | 42±9.37 | <.001 |
| Height in (C.M) | 158±11.77 | 163±7.7 | <.001 |
| Weight( in K.g) | 62±9.004 | 65±7.7 | <.01 |
| B.M.I( Ht/m2) | 25.04±3.29 | 25±2.71 | <.05 |
| Hip (C.M) | 101±7.94 | 96±8.26 | <.001 |
| Waist (C.M) | 94±8.52 | 86±8.22 | <.001 |
| W:H | .924±.033 | .881±.04 | <.001 |

**Table 2 :** Descriptive chemical characteristics of diabetic patients and controls

| Biochemical characteristics | Type-2 DM Mean±SE | Non-Diabetic controls mean±SE | P value |
|---|---|---|---|
| TAG | 226±124.16 | 138±89.23 | <.001 |
| Cholesterol | 192±42.11 | 165±33.06 | <.001 |
| LDL-C | 113±34.52 | 95±30.05 | <.001 |
| HDL-C | 37±5.49 | 41±9.003 | <.001 |
| HBA1C | 8.41±1.62 | 5±.330 | <.001 |
| Zn | .941±.246 | 1.21±.105 | <.001 |
| Cu | .771±.483 | 1.142±.239 | <.001 |
| Mg | 14±3.613 | 18±1.72 | <.001 |
| Mn | .091±.049 | .106±.030 | <.01 |

**Table 3 :** correlation of serum Mg, Zn, Cu, Mn concentration with lipid profile parameters in type-2 DM

| Lipid profile | Mg of type-2DM (r value) | Zn in Typ e-2 DM (r value) | Cu of type-2DM (r value) | Mn of type-2DM (r value) |
|---|---|---|---|---|
| TAG<br>P value | r =-.023<br>ns | r = .209<br><.05 | r=.049<br>ns | r=.090<br>ns |
| Cholesterol<br>P value | r =-.130<br>ns | r = .148<br>ns | r=.086<br>ns | r=.167<br>ns |
| HDL-C<br>P value | r =-.151<br>ns | r = .199<br><.05 | r= .051<br>ns | r=-.148<br>ns |
| LDL-C<br>P value | r =-.076$^{ns}$<br>ns | r =.004<br>ns | r=.129<br>ns | r=-.082<br>ns |

**Table 4 :** correlation of serum Mg, Zn, Mn, Cu concentration with lipid profile parameters in controls

| Lipid profile | Serum Mn | Serum Cu | Serum Mg | Serum Zn (r value) |
|---|---|---|---|---|
| TAG<br>P value | r=.191<br>p<.05 | r=.232<br>p<.05 | r =.260<br>p<.01 | r =.026<br>ns |
| Cholesterol<br>P value | r=.228<br>ns | r=.011<br>ns | r =.182<br>ns | r = .087<br>ns |
| HDL-C<br>P value | r =.120<br>ns | r =.060<br>ns | r =-.180<br>ns | r =.038<br>ns |
| LDL-C<br>P value | r=.045<br>ns | r=-.129<br>ns | r =.117<br>ns | r =.132<br>ns |

**Table 5 :** The effect of level of HBA1C in trace elements level at diabetic and Non diabetic patients

| Parameters | HBA1C | | T test |
|---|---|---|---|
| | Diabetic | Non Diabetic | |
| Zn | 5.819±.052 | .045±.003 | Ns |
| Cu | 2.035±.149 | 2.492±.138 | Ns |
| Mg | .297±2.004 | .405±.892 | Ns |
| Mn | .287±.004 | .512±.017 | Ns |

**Discussion**

Type 2 DM is a major global health problem that affects 200 million individual worldwide[7]. It is characterized by insulin resistance in peripheral tissues and an insulin secretory defect of beta cells of the pancreas[11]. The relationship of DM with minerals has been reported1-5. Among these minerals copper and magnesium are of particular interest. In the present study we obtained a significant increase in serum copper level in patients having type 2 DM as compared to controls. Zargar HA et al showed that copper levels were significantly elevated in NIDDM patients than in non diabetic subjects4. In a study done by Schlienger et al, elevated levels of copper were found in patients with IDDM and NIDDM[12]. Sarkar A et al, also found out a significant increase in serum level of copper in type 2 DM as compared to controls1. It is well known that copper plays a vital role in oxidative stress[1, 2]. Copper in its free form is a potent cytotoxic element because of its redox chemistry. It readily participates in Fenton and Heiber Weiss reactions to generate reactive oxygen species[13,14]. A high level of copper enhances the toxic effect of metal dependent free radicals. Moreover the increase in copper levels in patients with type-2 DM might also be attributed to hyperglycaemia, which stimulates glycation and causes release of copper ions from copper binding sites of proteins. The release of copper ions into blood further accelerates the oxidative stress 15. The other finding of this study was a significant decrease in serum magnesium level in type 2 DM as compared to controls. Similar such decrease in serum magnesium level in diabetics patients as compared to controls has been reported by some authors[2-4,16]. Magnesium is a cofactor for several enzymes involved in carbohydrate metabolism[17]. Magnesium is important for the effectiveness of insulin. It is involved at multiple levels in insulin secretion, binding and its activity. A reduction of magnesium in the cells strengthens insulin resistance[18,19]. Magnesium deficiency decreases insulin sensitivity via alteration of the insulin receptor associated tyrosine kinase in type 2 DM patients[17]. Hypomagnesaemia can increase the platelet reactivity, increase vascular and adrenal responses to angiotensin II enhanced thromboxane A2 release and lead to organ damage from free radicals[20,21]. Magnesium itself has been reported to possess antioxidant properties by scavenging oxygen radicals probably by affecting the rate of spontaneous dismutation of superoxide anions. Increased free radical formation and reduction in antioxidant potential contributes to the development of oxidative stress in type 2 DM4. The cause of hypomagnesemia may be attributed

to osmotic renal loss from glycosuria, and also decrease in net tubular reabsorption of magnesium[22]. The present study showed a significant ($p<0.001$) rise in HbA1c level in cases as compared to controls, which is similar to the findings of other studies[2,5,22]. The patients with DM who had altered metabolism of copper and magnesium were probably related to increase in HbA1c. 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 the insulin's ability to bind to its receptor. A decrease in zinc affects the ability of the islet cells to produce and secrete insulin, which could compound the problems of Type 2 diabetics in particular. In addition to the findings that zinc levels are often low in diabetics, it is also felt that zinc (in concert with other micronutrients) may participate as an integral component of antioxidant enzymes. Many of the complication of diabetes may relate to an increase in intracellular oxidant and free radicals associated with decrease in intracellular zinc and zinc dependent antioxidant enzyme[23]. Although this study shows a decrease in zinc with the age yet this was not significant and this consistent with results obtained by many studies, which showed that the elderly are at particular risk of zinc deficiency due to their low energy intake and poor dietary zinc consumption[22].

**Conclusion**

The present findings demonstrate the imbalance in levels of serum copper and serum magnesium among the patients of type 2 DM in comparison to controls. These changes may play an important role in the pathogenesis of type 2 DM by the involvement of these elements in the oxidative stress. Moreover increased levels of copper and decreased level of magnesium are associated with increased values of HbA1c. This suggests that the impaired metabolism of these minerals may have a contributory role in the progression of DM and later development of complications.

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