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Assessment Of Some Major Minerals And Trace Elements Levels In Female Patients With Type II Diabetes Mellitus And Their Correlation With Glycated Hemoglobin

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ABSTRACT

The metabolism of several minerals and trace elements has been reported to be altered in diabetes mellitus which might have specific roles in the pathogenesis and progress of this disease. Objective: the aim of the present study was to investigate plasma levels of calcium, phosphorus, magnesium, copper, zinc and iron in type II diabetic female patients and their possible associations with glycemic status. Methodology: A cross sectional comparative study was carried out on 61 type II female diabetic patients besides to 49 non-diabetic subjects with matched age, sex and social level. Blood samples were withdrawn for spectrophotometerical determination of the levels of fasting glucose, glycated hemoglobin (Hb_{A1c}), trace elements (copper, zinc and iron) and minerals (magnesium, phosphorus and calcium). Results: Both calcium and phosphorus levels in type II diabetics were significantly ($P<0.05$) different compared to non-diabetic healthy control group; calcium decreased while phosphorus increased. On the other hand, levels of plasma magnesium, copper, zinc and iron were insignificantly ($p>0.05$) different in diabetic patients in comparison with control subjects Hb_{A1c}% recorded a significant ($p<0.01$) positive correlation with both fasting glucose and iron levels in diabetic female patients ($r = 0.445, 0.434$) respectively, while no significant ($p>0.05$) correlation was detected with magnesium, copper, phosphorus, calcium and zinc levels. A significant ($p<0.05$) negative correlation was observed between calcium and magnesium, zinc, duration of the disease and patients' age ($r= - 0.347, -0.421, - 0.282, - 0.301$) respectively. Copper level performed a significant ($p>0.05$) positive correlation with phosphorus, duration of the disease, fasting blood glucose levels, age and magnesium ($r= 0.454, 0.346, 0.295$) respectively, while no correlation between zinc and iron was detected in diabetic patients. Conclusion: The results denoting that glycemic status may affect the trace elements concentrations, also it confirms that some minerals and trace elements altered in diabetics may play a role in the development of diabetes or diabetic complications.

Key words: Diabetes, minerals, trace elements, free radicals, oxidative stress.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by high blood glucose levels, which result from defects in insulin secretion, action, or both (Choudhury and Sanyal, 2004).There has been accumulating evidence that the metabolism of minerals is altered in diabetes mellitus and that those nutrients might have specific roles in the pathogenesis and progression of this disease or metabolic complications (Muhittin *et al*., 2009).

In pancreatic beta cells, Ca^{++} influx plays a critical role in regulating glucose stimulated insulin secretion (GSIS) that is the major mechanism for insulin release. A study revealed that the level of serum Ca was significantly and positively correlated with the levels of fasting plasma glucose, immunoreactive insulin, and homeostasis model assessment insulin resistance in men, but not in women. In contrast, intact parathyroid hormone (PTH) was not significantly correlated with DM-related parameters in either sex (Yamaguchi *et al*., 2011). Phosphorus is a key element in all known forms of life; living cells also use phosphate to transport cellular energy in the form of adenosine triphosphate (ATP). Nearly every cellular process that uses energy, obtains it in the form of ATP which is so important for phosphorylation, a key regulatory event in cells. Phospholipids are the main structural components of all cellular membranes and calcium phosphate salts assist in stiffening bones (Paytan and McLaughlin, 2011).

Alterations in bone mineral content (BMC) have been reported in type II diabetes mellitus. In poorly controlled diabetes, the loss of BMC is aggravated by the negative calcium balance caused by the renal calcium leak. This is due to glucosuric-induced osmotic diuresis and is maintained by parathyroid activation (Gregorio *et al*., 1994). Improvement of hyperglycemia may alter calcium and phosphorus handling, PTH secretion and bone turnover in patients with type II diabetes mellitus (Nagasaka *et al*., 1995).

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Magnesium, the fourth most abundant cation in the body, fulfils various intracellular functions. It plays an important role in numerous enzymatic reactions especially in ATP generating reactions, antagonizes calcium in muscle contraction, modulates insulin signal transduction and cell proliferation, and is important for cell adhesion and membrane transport (Jahnen-Dechent *et al*., 2012). Magnesium is an important cofactor in several enzymes critical for carbohydrate metabolism and is believed to play a role in glucose homeostasis, insulin action, and the development of type II diabetes (Saris *et al*., 2000; Barbagallo *et al*., 2003). The previous studies those related to magnesium levels in diabetics are inconsistent as many studies showed no significant difference in serum magnesium level of diabetic patients when compared with that of control subjects (Masood *et al*., 2009). In animal models, Mg deficiency results in many consequences including increased serum glucose, decreased glucose utilization, insulin release, reduced insulin sensitivity and decreased phosphorylation of the β-subunit of the insulin receptor, matched with decreased tyrosine kinase activity (Barbagallo *et al*., 2003).

The role of trace elements for improvement of disturbed metabolic conditions like pre-diabetes and diabetes has been gaining attention (Valko *et al*., 2005; Zheng *et al*., 2008). Zinc (Zn) is essential for the formation of both stored and active form of insulin (Rutter, 2010). It may also be responsible for the conformational changes that allow insulin to bind to its receptors for activity. In addition, it has been suggested that zinc may be involved in the development or progression of both type 1 and type 2 diabetes (Prabodh *et al*., 2011; Kazi *et al*., 2008). Zinc plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells. Intestinal zinc absorption rates and plasma zinc levels in diabetic patients are reduced (Song *et al*., 1998). Zn is considered important as it plays a major role in the stabilization of insulin hexamers and the pancreatic storage of the hormone (Wijesekara *et al*., 2009). Prasad (2008) found that it is an efficient antioxidant, while Kaneto *et al*. (2010) and Wiernsperger (2003) stated that oxidative stress is considered to be a main component in initiation and progression of insulin resistance and diabetes.

Copper (Cu) is the third most abundant mineral in the human body (Masood *et al*., 2009). It is an essential component of metalloenzymes such as, Cu/Fe cytochrome C oxidase and superoxide dismutase (SOD), those have an important role in both intra- and extra-cellular antioxidant defense (Zheng *et al*., 2008; Beckett and Arthur, 2005). Moreover, Cu and Zn are the major components of the antioxidant enzyme SOD (Yamaguchi *et al*., 2011) which is greatly involved in the inhibition of oxidative stress resulting from the accumulation of free radicals and plays an important role in the pathological processes ongoing in diabetic patients. Deficiency of essential elements may lead to failure of the antioxidant defense and also to glucose intolerance. Both are important in the progress of diabetes. Furthermore, the overload of some transition metals as redox-cycling metal copper Cu (I)/Cu (II) may be responsible for oxidative damage (Viktorínová *et al*., 2009).

The importance of iron (Fe) in diabetes pathophysiology is explained by catalytic iron or iron that is available to participate in free radical reactions. Excessive Fe intake was found to give rise to increased risk of insulin resistance and diabetes whereas excess amounts of Cu and Fe were also found to significantly increase the incidence of diabetic complications. Free radical production is considered as one of the major mechanisms responsible for the toxicity of Cu and Fe. Superoxide radicals generated in all aerobic cells have been attributed to the Fe- and Cu-dependent formation of reactive oxygen species (ROS) through Fenton's reaction. These ROS including hydroperoxyl radicals, singlet oxygen, and hydroxyl radicals are highly reactive and can cause damage to biomolecules. On the other hand, low concentrations of these ROS are required as they act as second messengers, gene regulators, and are even involved in insulin signaling (Cai. 2006; Galaris and Pantopoulos, 2008).

Glycated hemoglobin (HbA_{1c}) is an important indicator of glycemic control. HbA_{1c} is a laboratory test that shows the average amount of blood sugar over the previous 3months prior to the measurement. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. HbA_{1c} levels being used to diagnose diabetes are less than 5.7% in normal, 5.7% to 6.4% in pre-diabetes, and 6.5% or higher in diabetes (American Diabetes Association, 2011).

It is not always clear whether diabetes mellitus and hyperglycemia affect mineral metabolism or alterations in mineral homeostasis influence carbohydrate metabolism (Valko *et al*., 2005); therefore, the aim of the present study was to investigate plasma levels of some minerals (including: calcium, phosphorus and magnesium) as well as plasma levels of trace elements (copper, zinc and iron) in type II diabetic female patients and their possible associations with glycemic status.

Methodology and Subjects:

This study was conducted on two groups; the first group comprised of 61 female patients with diabetes mellitus type II (duration 11.30 ± 7.49 years) enrolled from Prince Maged Ben Abdel Aziz diabetic Center, Al Madina, KSA; the second group comprised of 49 healthy non diabetic females, from Taibah university students and staff of matched age and social level, and acted as control group. Both groups haven't received any supplement medications. Blood samples (4 ml) from both diabetic and healthy individuals were collected (2ml in heparinized tube and the other 2ml in an EDTA tube). The heparinized portion was centrifuged at 3000 rpm

for 5 minutes; and the plasma was separated in aliquots for investigation of the plasma levels of glucose, trace elements (copper, zinc and iron), the minerals (magnesium, phosphorus and calcium) and albumin for correction of abnormal calcium level. The EDTA anticoagulated blood samples were used in determination of glycated hemoglobin level (HbA_{1c}), the percentage of blood HbA_{1c} was determined by Nycocard HbA_{1c} Reader. Using spectrophotometry, plasma glucose concentration was estimated without deproteiniation using the kit purchased from Human Gesellschaft, Germany). Plasma levels of the major elements (magnesium, phosphorus) were evaluated with lipid clearing factor (LCF) using Human Gesellschaft kit, Germany, while the level of calcium was determined according to the CPC method using the kit purchased from Human Gesellschaft.

The plasma levels of the trace elements copper, zinc and iron were determined colorimetrically using QCA (QUIMICA CLINICA APLICADA, K.S.A.) kits. A colorimetric determination of iron was carried out with lipid clearing factor (LCF) using kits purchased from Human Gesellschaft, Germany. Similarly, plasma albumin level was estimated using kits of Human Gesellschaft, Germany. The plasma level of the trace element, copper, was determined colorimetrically using kits purchased from Randox, United Kingdom. Ultimately, all the obtained data were subjected to statistical analysis using SPSS for windows (version 16.0, SPSS, Chicago, IL, USA). Student's *t* test was used for comparison of the baseline continuous characteristics between the two groups (control and type-2 diabetes). Given the small sample size, the correlations were found using the Spearman correlation; $p < 0.05$ was considered to be statistically significant.

Results:

The obtained results showed that both calcium and phosphorus plasma levels were significantly different in type II diabetics compared to non-diabetic healthy control group, P<0.05, (Table 1 and figure 1), as calcium concentration decreased significantly while phosphorus increased significantly when compared with the control group. On the other hand, plasma magnesium levels were not significantly different in type II diabetics when compared to non-diabetic healthy control group, P>0.05 (Table 1 and figure 1).

Table 1: Mean values of glycated hemoglobin (HbA1c), magnesium (Mg), copper (Cu), phosphorus (P), calcium (Ca), iron (Fe), zinc (Zn), age of both diabetic and healthy non–diabetic subjects.

Parameter	Control	Diabetic	p- value
Mg (mg/dl)	2.33 ± 0.14	2.37 ± 0.10	P>0.05
Cu (µg/dl)	123.54 ± 27.44	124.31 ± 19.46	P>0.05
P (mg/dl)	3.79 ± 0.89	4.40 ± 1.01*	P<0.05
Ca (mg/dl)	9.41± 1.08	9.03 ± 1.16*	P<0.05
Fe (µg/dl)	55.77± 23.71	58.63±24.69	P>0.05
Zn (µg/dl)	128.84± 73.41	96.54±16.65	P>0.05
HbA1c (%)	5.38 ± 0.55	8.90 ±1.78**	P<0.01
Glucose (mg/dl)	90±8.2	189.7 ± 78.97**	P<0.01
Age (year)	48.46+15.12	52.91 ± 10.11	P>0.05

All data are presented as mean± SD, * p<0.05, ** p<0.01.

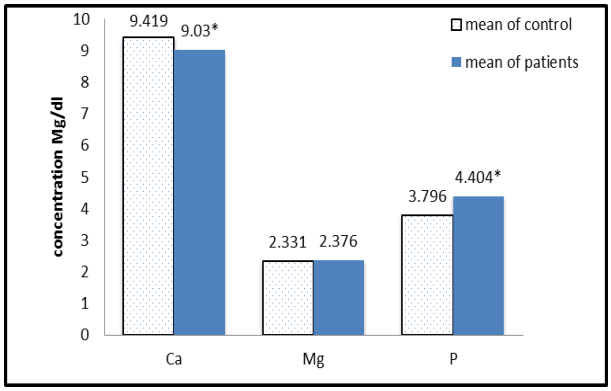

Fig. 1: Mean values of plasma level of magnesium, phosphorus and calcium of diabetic and control groups. (* p<0.05).

The results also illustrated that in type II diabetic female patients there was a significant positive correlation between glycated hemoglobin and fasting glucose levels, p<0.05 (r = 0.462) (Table 2 and figure 4), while there was no significant correlation between glycated hemoglobin with magnesium, phosphorus, calcium, copper and zinc; P>0.05 (Table 2). In type II diabetic female patients, an inverse significant correlation between age and plasma calcium is observed (r = - 0.301) (P<0.05) (Table 2 and Figure 7), while there is a significant positive correlation between age and magnesium (r = 0.295, P<0.05) as shown in table 2 and figure 8. However, there was no correlation found between age and copper, zinc, phosphorus or iron (p>0.05) (Table 2).

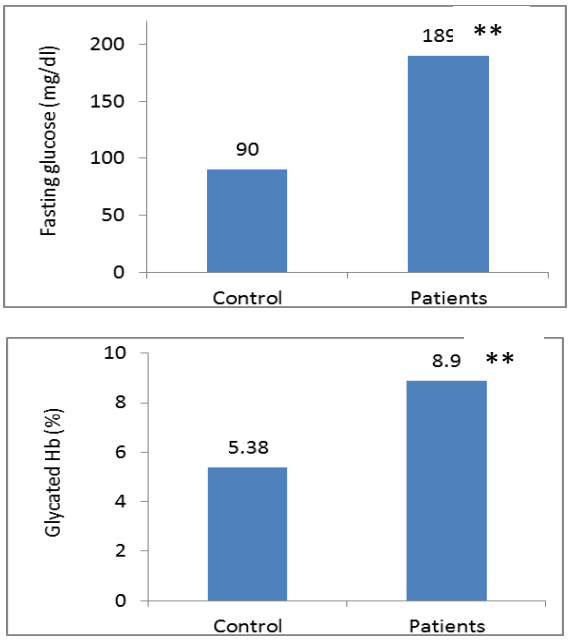

Fig. 2: Means values of fasting blood glucose (a) and HbA1c (b) levels of diabetics and control group (**p<0.01).

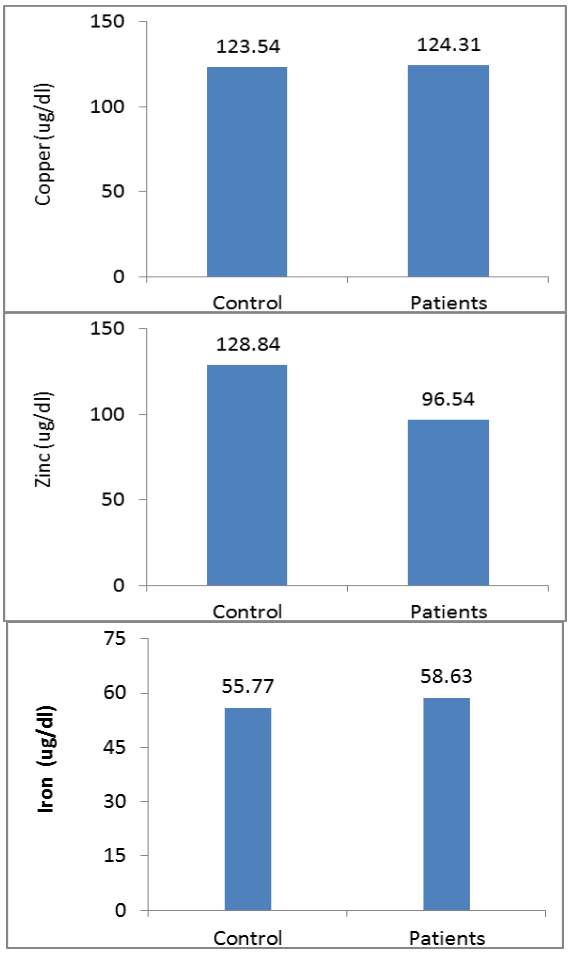

Fig. 3: Mean values of plasma levels of iron, zinc and copper of both diabetic and control groups.

Table 2: Correlation between different minerals (Zn, Fe, Mg, Cu, P and Ca), HbA1c, disease duration, age and fasting glucose in diabetic patients.

Variables	Mg (mg/dl)	Cu (µg/dl)	P (mg/dl)	Ca (mg/dl)	Glu (mg/dl)	Fe (µg/dl)	Zn (µg/dl)
Age (y)	r = 0.295*	r = -0.214	r = - 0.059	r =- 0.301*	r = 0.036	r =0.021	r = -0.072
Duration (y)	r = 0.125	r = 0.057	r = - 0.055	r =- 0.282*	r = 0.346*	r =0.046	r =-0.027
HbA1c (%)	r = 0.002	r = 0.177	r = 0.049	r = - 0.143	r= 0.462**	r = 0.43*	r = -0.237
Zn (µg/dl)	r = -0.230	r = -0.226	r = 0.113	r = -0.421*	r= -0.218	r =-0.13	
Fe (µg/dl)	r = -0.104	r = 0.245	r = 0.026	r = 0.103	r= -0.252		
Glu (mg/dl)	r = 0.109	r = 0.227	r = - 0.064	r = - 0.232			
Ca (mg/dl)	r = -0.347*	r = 0.188	r = - 0.024				
P (mg/dl)	r=0356	r= 0.454**					
Cu (µg/dl)	r = - 0.205						

* P<0.05, **P<0.01

A significant positive correlation was observed in diabetic female patients between fasting glucose levels and duration of the disease (r = 0.346, P<0.05) (Table 2 and Figure 5), but an inverse significant correlation between calcium levels and duration of the disease (r = - 0.282, *p*<0.05) (Table 2 and Figure 6), while there was no correlation between duration of the disease and magnesium, copper and phosphorus (*p*>0.05) (Table 2).

The results also showed that in type II diabetic female patients there was an inverse significant correlation between magnesium and calcium (r = -0.347, *p*<0.05) (Table 2 and Figure 9) but a significant positive correlation between copper and phosphorus (r = 0.454, *p*<0.05) was noticed (Table 2 and Figure 10). Regarding trace elements, the results showed that none of the trace elements; plasma iron, zinc or copper levels were significantly different in type II diabetics compared to non-diabetic healthy control group, *p*>0.05 (Table 1 and Figure 3). In addition, the obtained results showed that there was a significant positive correlation (*p*<0.05) between plasma iron and glycated hemoglobin (r =0.434); while there was no correlation between zinc and iron levels in type II diabetic female patients (Table 2), however, there was a significant inverse correlation between zinc and calcium levels in diabetics (Table 2 and Figure 11).

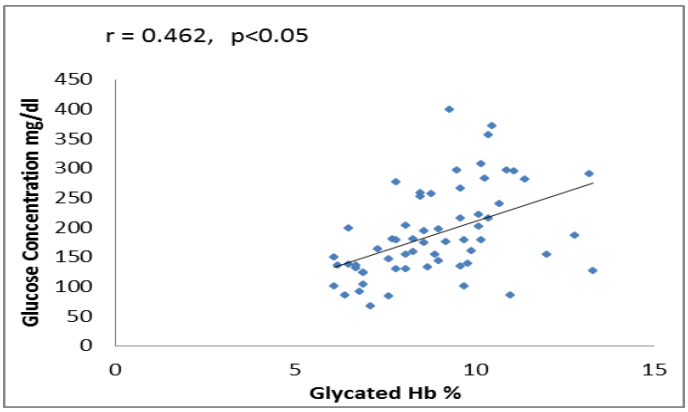

Fig. 4: Significant positive correlation was noticed between glycated hemoglobin (HbA1c) and fasting glucose of diabetic patients (r = 0.462, *p*<0.05).

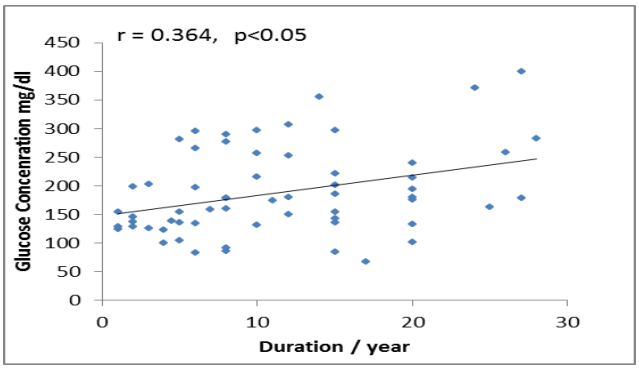

Fig. 5: Significant positive correlation was detected between duration of diabetes and fasting glucose level in diabetic patients (r = 0.364, *p*<0.05).

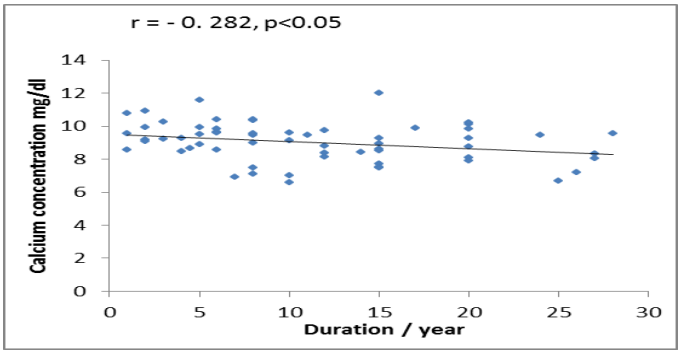

Fig. 6: A significant inverse correlation was observed between duration of diabetes and calcium (Ca) concentration in diabetic patients (r = -0.282, p<0.05).

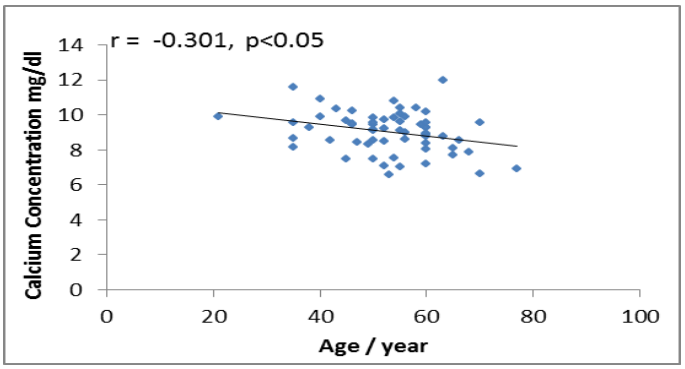

Fig. 7: Age of diabetic subjects showed significant inverse correlation (r = -0.301, p <0.05) with calcium concentration in diabetic patients.

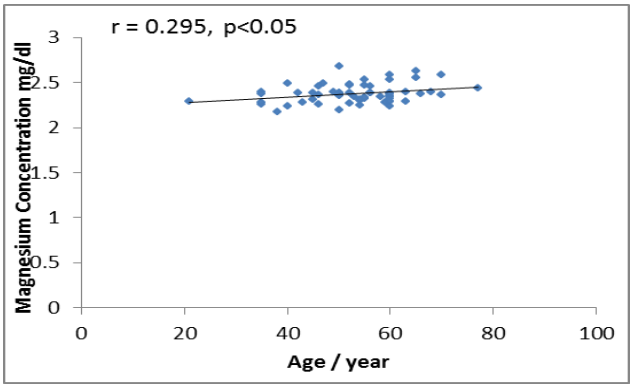

Fig. 8: Age of the patients recorded a significant positive correlation (r = 0.295, p<0.05) with magnesium (Mg) concentration in diabetic patients.

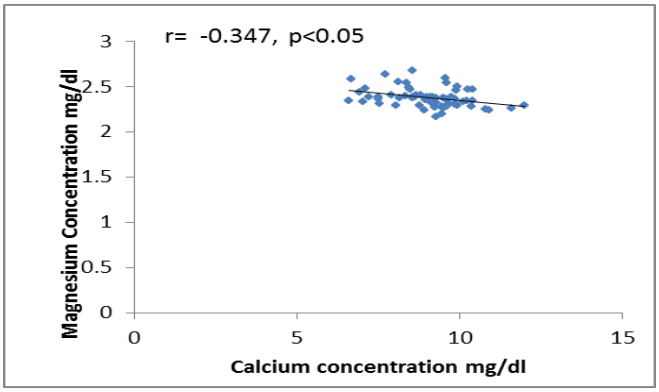

Fig. 9: Magnesium (Mg) and calcium (Ca) concentration of diabetic patients showed a significant inverse correlation (r= -0.347, p<0.05) with each other.

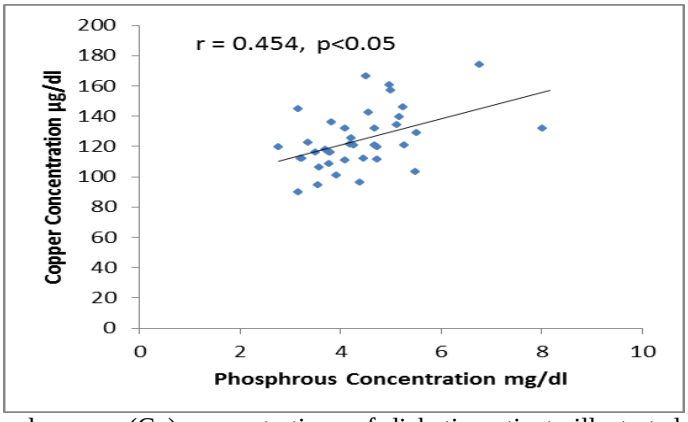

Fig. 10: Phosphorus (P) and copper (Cu) concentrations of diabetic patients illustrated a significant positive correlation (r = 0.454, P<0.05) with each other.

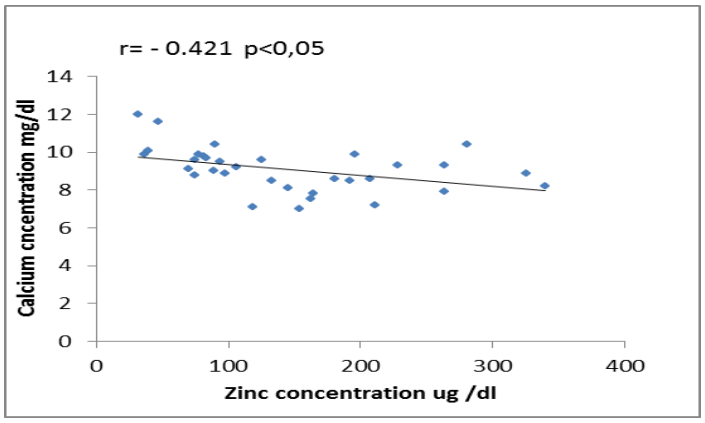

Fig. 11: Zinc and calcium (Ca) concentrations of diabetic patients showed a significant inverse correlation (r= -0.421, p<0.05).

Discussion:

Diabetes mellitus is the most common cause of end-stage renal disease, accounting for about 40% of cases in many countries. As diabetics have an increased risk of developing renal insufficiency as well as congestive heart failure, independent of hypertensive and coronary atherosclerotic disease (Zheng *et al*., 2008); therefore the specific aim of this study was to pursue possible relationships between serum concentration levels of several micronutrients and trace elements as typical indicators used in the follow-up of diabetic patients, thus contributing to better understanding of the global role of major and trace elements in type II diabetes mellitus complications.

The obtained results revealed lower plasma calcium matched with higher phosphorus levels in diabetic patients of this study; whole phosphorus plasma level differed relatively when the patients were poor in metabolic control. Controversially, magnesium concentration remained within the normal range of that of control persons. Also, it seems that there was no magnesium deficiency as well as no significant change in Copper level of our diabetic patients. These results are in agreement with the findings of Durak *et al*. (2010). Ca and Cu concentrations in both diabetic patients (with and without complication) were significantly lower than those in healthy controls; while level of Mg differs in relation to complication when compared with non-diabetic control group; the Mg concentrations of patients without complication were decreased, while its level of patients with at least one complication was increased as expected in diabetic nephropathy; renal failure is commonly associated with hypermagnesaemia (Swaminathan, 2003)**.** Causes of poor renal excretion those affect mineral as magnesium include advancing age which could tend to reduce renal function and also diabetic ketoacidosis (DKA). On the other hand, decreased magnesium which occurs in diabetic patient without complication may be a result of osmotic diuresis (Håglin *et al*., 2007). This can explain our result as some of the patients of this study were had diabetes for many years with renal involvement, while others were recently diagnosed with controlled diabetes.

The indifferences in the magnesium levels in this study is similar to the results reported by Zargar *et al*. (1998) and Masood *et al*. (2009) who found no statistical significant difference regarding magnesium level

between both diabetic and control groups; also, they reported a significant correlation between age and magnesium level which was significantly higher in younger ages (< 45 years) of diabetic patient.

The significant increased phosphorus in our finding is in agreement with the result of Kim *et al*. (2007) who reported that level of phosphorus in the aqueous humor and serum of diabetics was significantly increased, especially in diabetics with proliferative diabetic retinopathy. This result may be related to hydrophilic acrylic intraocular lens pacification. The pathogenesis of diabetic late complications (DLC) is multifactorial. Studies of mechanisms leading to early functional micro vascular changes in retina and kidneys pointed towards a disturbance in the metabolism of inorganic phosphate (Pi) in diabetes, since tissue hypoxia and reduced high energy phosphates may be important factors in the development of DLC. The more prevalent form, type-2 diabetes, affects approximately ≥ 10% of the general population (with substantially higher rates at ≥55 years of age) and is well known as an independent predictor of accelerated decline in kidney function (Ford, 2001). Moreover, our findings herein, regarding both calcium and phosphorus are parallel with the data published by Zywiec *et al*. (2001) who suggested a tendency to early disturbances of calcium-phosphorus metabolism in both type I and II diabetic subjects with good metabolic compensation and without advanced diabetic complications.

Improvement of hyperglycemia may alter calcium and phosphorus handling, parathyroid hormone (PTH) secretion, and bone turnover in patients with type II diabetes mellitus as elevated serum phosphorus, increased urinary calcium and decreased phosphorus excretion.

Diabetic nephropathy aggravates derangement of phosphoric-calcium metabolism in patients with diabetes mellitus. Secondary hyperparathyroidism, as a result of negative calcium balance in decompensated diabetes mellitus and renal dysfunction, contributes to development of marked alterations of the osteoarticular system (Khasanova *et al*., 1991). Patients with chronic kidney disease (CKD) have loss of kidney function that, in many cases, progresses to end-stage renal disease. For each 1 mg/dL increase in phosphorus level, the risk of coronary artery calcification increased by 21 percent, after adjustment for level of kidney function and other characteristics. Phosphate-lowering drugs, generally used only in patients with end-stage renal disease who have higher than normal phosphorus levels, might help to reduce cardiovascular risk in CKD patients and even in healthy adults with high-normal phosphate levels (Jean, 2011).

Excess phosphorus can combine with calcium forming salts which can deposited in tissue causing hypocalcaemia; hypocalcaemia occurred when parathyroid hormones is eliminated or reduced. Renal failure is associated with insufficient vitamin D activation leading to hypocalcaemia which also can occur due to excess excretion of calcium due to osmotic diuresis (Kestenbaum *et al*., 2007).

Vitamin D and calcium insufficiency may negatively influence glycaemia, while combined supplementation with both nutrients may be beneficial in optimizing glucose metabolism. An association between low vitamin D status and calcium intake, including low dairy intake, as well as risk of type II diabetes mellitus or metabolic syndrome was reported by Anastassios *et al*. (2007).

Also this study showed an agreement with the result published by Hussain *et al*. (2009) as the difference observed in Cu level was not significant between diabetic and normal subjects. In addition, glycemic status, duration of diabetes and age did not affect the Cu concentrations. The redox reaction of transition element Cu (Cu^{+}/ Cu^{++}) resulted in both powerful enzyme catalyst and dangerous reactant that generates hydroxyl radical. Although virtually all cells from microbes to mammals must acquire Cu to drive important biochemical reactions, the potential toxicity of Cu demands an exquisite level of vectorial transport and homeostatic control. Abnormal copper metabolism can lead to several chronic pathogenesis, such as diabetes or diabetic complications.

The obtained data of our study are in agreement also with that carried out by Prabodh *et al*. (2011) and Kazi *et al*. (2008) who studied the level of essential trace elements, as Cu in whole blood, urine, and scalp hair, of patients who have type II diabetes mellitus; their results showed elevated Cu levels in scalp hair and blood of diabetic patients compared to those of non-diabetic subjects, but the difference found in blood samples was not significant ($P > 0.05$).

Tanaka *et al*. (2009) reported that the mean concentrations of fasting blood glucose (FBS), post prandial blood sugar (PPBS), glycated hemoglobin (HbA1c), and microalbuminuria of diabetic cases were significantly higher than that of healthy controls, but the mean copper levels of cases showed non significant difference from controls. These findings are consistent with our data. The importance of Cu in diabetes pathophysiology is that Cu under a non protein-binding condition can generate oxygen species, through the Fenton reaction mediated generation of hydroxyl radical with damaging effects on tissues or cells. After treatment with a copper chelating agent, both serum copper ions and reactive oxygen species (ROS) levels were significantly decreased as well as insulin resistance and glucose intolerance were reduced.

This study evidenced that age has significant positive correlation with magnesium level, matched with no correlation with copper level in diabetic females. This finding is in agreement partially regarding copper with Zargar *et al*. (1998) who suggested that the age did not influence copper or magnesium concentrations. Also no influence was found regarding the duration of the disease on plasma copper, and magnesium concentrations as stated in our study. Additionally, insignificant association of copper levels with HbA1c levels was found in our

study. Walter *et al*. (1991) illustrated that plasma copper level was higher in diabetic subjects with retinopathy, hypertension, macrovascular, macro- and microangiopathy and/or altered lipid metabolism in comparison either with no complications diabetic subjects or with control subjects.

Although no difference was noticed between the diabetic and healthy groups regarding the plasma levels of iron, zinc and copper; the strong correlation between plasma iron and HbA1c levels may indicate an underlying analogy between diabetic complications and this element. In this study, our data revealed that plasma iron level was higher while that of zinc was lower in diabetics than in control group, however these differences were statistically insignificant ($P>0.05$) and this insignificance may be related to the small number of patients investigated. A lot of investigations tried to explain the alteration which occurs in uncontrolled diabetic patients in mineral metabolism. Liu *et al*. (2009), explained the roles of iron deficiency and overload in pathogenesis of diabetes and its complications as well as the possible links of iron to diabetes and diabetic complications. They summarized that as iron is one of the essential minerals that are required for a variety of molecules to maintain their normal structures and functions as well as for cells to live, grow, and proliferate; the homeostasis of iron results from a tightly coordinated regulation by different proteins involved in uptake, excretion and intracellular storage/trafficking; and although it is essential, iron can also be toxic once in excess amounts.

Through a Fenton reaction, iron as a transition element can generate various reactive oxygen or nitrogen species; therefore, abnormal metabolism of iron can lead to several chronic pathogenesis. Oxidative stress is one of the major causative factors for diabetes and diabetic complications. Increasing evidence has indicated that iron overload not only increases risks of insulin resistance and diabetes, but also causes cardiovascular diseases in both non-diabetic and diabetic subjects. Temporal iron deficiency was found to sensitize insulin action, but chronic iron deficiency with anemia can accelerate the development of cardiovascular diseases in non-diabetic and diabetic patients (Serdar *et al*., 2009).

In this study, a significant positive correlation was observed between serum iron and glycated hemoglobin. Kim *et al*. (2011) suggested that iron overload is associated with insulin resistance in men, but not in women; they reported increased serum ferritin concentrations in non-pathological conditions, reflecting subclinical iron overload, have been reported to be associated with insulin resistance and an increased risk of type II diabetes mellitus. However, serum ferritin concentrations differ significantly according to sex and ethnicity; and data concerning the relationship between serum ferritin concentrations and glucose metabolism abnormalities in Asian men and women are conflicting. Increased serum concentrations of ferritin are associated with insulin resistance, type II DM; impair fasting glucose (IFG) and metabolic syndrome in men, but it is only associated with IFG in women.

Some studies suggested that poor glycemic control and increased glycation of hemoglobin are contributing to the increase in free iron pool which is known to increase oxidant generation, and enlarge microvascular complications of diabetes (Zheng *et al*., 2008). Previous studies have proved that poor glycemic control causes increased glycation of proteins, especially hemoglobin, which releases the iron in its free state; hence increased presence of free iron in its Fe^{3+} state in association with hyperglycemia might have caused a decrease in the levels of protein bound thiols and increase in lipid hydroperoxides. Studies have shown linear relationship between free iron and glycated hemoglobin in in-vitro experiments (Galaris and Pantopoulos, 2008).

Our study monitored that zinc decreased insignificantly in diabetics; this finding goes in parallel with that of Zheng *et al*. (2008) who concluded that diabetes was associated with Zn deficiency and probably also with Cu deficiency; consequently, zinc supplementation may provide a significant protection against diabetes induced complications for diabetic individuals (Hayee *et al*., 2005**).** Alterations in zinc homeostasis appear to be related to diabetes as hyperzincuria seems well documented in both type I and type II diabetes and may be influenced by both sex and glycaemic status (Kinlaw *et al*., 1983). Plasma zinc may be lowered in type II diabetes or elevated in type I diabetes (Jansen *et al*, 2009). It was reported that Zn deficiency has been linked to the diabetes mellitus in experimental and clinical studies (Taylor, 2005; Islam and Loots, 2007). In addition, experimental studies also showed that various Zn chelators induce diabetes in some mammalian species (rabbits, mice, and hamsters) through β-cell destruction; and Zn deficiency significantly enhances the blood glucose level in diabetes-prone experimental animals (Kechrid *et al*., 2001).The finding of cardiac protection by Zn supplementation was consistent with an early clinical observation in which a significant prevention of diabetes caused neuropathy was achieved in Zn-supplemented diabetic patients (Hayee *et al*., 2005).

Perturbations in mineral metabolism are more pronounced in diabetic populations with specific complication, it is not known whether differences in elements status are a consequence of diabetes, or alternatively, whether they contribute to the expression of the disease.

Conclusion:

In conclusion, patients with diabetes mellitus type II had altered metabolism of phosphorus and calcium, and this was more to relate with diabetic patients with complications, advanced age of the patients and prolonged duration of the disease. Age, duration and control of diabetes did not influence copper or zinc

concentrations; therefore the levels of trace elements, zinc and copper, are not altered in diabetes mellitus, but the increased phosphorus associated with decreased calcium levels found in diabetics may merit further investigation of the relationship between complications and mineral metabolism in type 2 diabetes mellitus.

Association was found between glycated hemoglobin and both iron and fasting blood sugar levels, but no association between the other studied minerals and glycated hemoglobin was declared. These results are consistent with those obtained in other studies confirming that metals may play significant roles in the development of diabetes mellitus and progression of its metabolic complications. Nevertheless, comprehensive studies covering larger populations are needed to elucidate a clear relationship between glucose metabolism disorders and serum levels of trace metals.

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