



Lipid Profile, Lipid Per-oxidation and Trace Elements Status in Libyan Males with Type II Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration between all authors. Author RA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors AAN, NA and SB managed the analyses of the study. Authors AAN and BS performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The metabolism of several trace elements is altered in diabetes mellitus (DM). The present study investigates serum levels of lipid profile and lipid per-oxidation as well as levels of Mg, Cu, Ni, Co, Mn, Cr, Se, V and Zn, in 72 males with non- insulin- dependent diabetes mellitus (T2DM) and 21 non-diabetic healthy control subjects using inductively coupled plasma optical emission spectrometry (ICP-OES). The results showed highly significant increase in serum concentrations of LDL-C, TG and Cholesterol in T2DM patients in comparison with non-diabetic subjects ($P<0.001$). The levels of Zn, Mg and V in male diabetic patients showed significant decline as compared to controls ($P<0.001$). Also serum Cr and Co showed a significant decrease between non-diabetic subjects and T2DM patients ($P<0.05$), whereas Ni, Mn and Se showed no significant differences

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between the control and T2DM patients. The serum Cu level revealed a substantial increase in T2DM patients compared to non-diabetic individuals ($P<0.001$). Therefore, deficiencies in trace elements and high level of lipid per-oxidation products appear to be possible additional risk factors in the some pathogenesis of type-2 diabetes mellitus and its complications. In addition, they could be used as markers to evaluate the glycemic control and the lipid status of diabetic patients.

Keywords: Diabetes; trace elements; lipid per-oxidation; ICP-OES.

1. INTRODUCTION

Diabetes Mellitus (DM) is a chronic disorder of carbohydrates, lipids, and proteins metabolism. It is characterized by an absolute or relative deficiency of insulin and/or the absence of insulin receptors [1,2].

Changes in the concentration of insulin have also been shown to influence or to be associated with changes in the concentrations of several trace metals in the blood, urine and other tissues [3].

Trace elements are essential nutrients with regulatory, immunologic, and antioxidant functions as they act as essential components or cofactors of some enzymes throughout metabolism [4]. Each trace element contributes differently in many important physiological and biochemical processes in the body. The interaction between trace elements in biological processes play a role in mediating biological and chemical reactions which could be applied to the management of human health [5-7]. A significant relationship was found between trace elements status and different DM states [8].

Numerous studies have clearly demonstrated that some trace elements are involved in regulating or potentiating insulin action [9,10,7]. Changes in blood insulin levels have also been shown to influence or to be associated with changes in the concentrations of several trace metals in blood, urine and other tissues [3]. Establishing links between trace element concentration in blood and diabetes can thus be a useful mean to predict and manage diabetes complications [10,11,2]. Complications of diabetes are usually associated with increased production of free radicals or impaired antioxidant defenses [12,13]. For instance the decreased levels of selenium, zinc and copper may affect antioxidant system in diabetes [14].

DM may alter the copper, zinc, chromium, magnesium and lipid per-oxidation status [15]. Therefore, the aim of the present study was to investigate lipid profile, lipid per-oxidation and the

levels of Mg, Cu, Ni, Co, Mn, Cr, Se, V and Zn in Libyans with T2DM compared with healthy group as well as the correlation between levels of trace elements and lipid profile content was also examined.

2. MATERIALS AND METHODS

2.1 Ethical Considerations

The protocol of the study was approved by the Ethical committee of Biotechnology Research Center (Tweasha, Tripoli, Libya) on 2014. In addition, the study was performed according to the guidelines present in the Declaration of Helsinki which was amended in 2013 [16]. Informed consent was sought and obtained from individuals before enrollment into the study.

2.2 Design of the Study (Subject, Groups and Samples)

This study was a cross-sectional study conducted on 93 subjects (72 with T2DM and 21 controls) who attended the Endocrinology & Diabetes Center (Tripoli, Libya), with age ranging from 30 to 70 years. Subjects included in the current study were selected from the general population according to the following criteria: first, they were suffering and diagnosed as T2DM patients. Second, they were free of any ailment which could affect the parameters under study, and third, they are not on any medication. Patients with type-1 diabetes mellitus, hemolytic anemia, hemoglobin variants, pregnancy, hepatic disease and infectious diseases like tuberculosis, and sarcoidosis, were excluded from this study. In addition, subjects under treatment with drugs such as chelating agents, and ethambutol, D-penicillamine were also excluded. Twenty one apparently healthy, non diabetic subjects of similar socioeconomic status, who were members of the hospital community, were recruited to serve as control. A detailed medical history was taken and a physical examination was performed upon all participants.

2.3 Blood Sample Collection

Blood samples were taken after an overnight fasting and collected into special metal-free tubes for analysis of trace elements and biochemical parameters. Blood samples (10 ml) were obtained from the capital vein of each participant using sterile disposable plastic syringe. Specimens were collected at standardized time to minimize any effect of diurnal variation. Blood samples in a vacutainer tubes left to clot and the serum was separated by centrifugation at 4°C.

2.4 Determination of Biochemical Parameters

Various biochemical parameters in blood including: Fasting blood sugar (FBS) measured by the method of (Bondar and Mead, 1974) [17], triglycerides (TG) [18,19], total cholesterol (TC) [20], high-density lipoprotein cholesterol (HDL-C) [21], low-density lipoprotein cholesterol (LDL-C) and Glycosylated hemoglobin (HbA_{1c}) according to International Federation of Clinical Chemistry (IFCC) [22,23].

All biochemical parameters measured according to standard protocols using Pre-modular analytics by using a kit with COBAS INTEGRA® 400 plus in the Biochemistry Laboratory, at the Pathology Department in the Diabetes & Endocrinology Center, Tripoli, Libya.

2.5 Determination of Lipid Per-oxidation Level in Serum

Serum lipid per-oxidation was measured according to the method described by Burtis and Ashwood, [24]. The levels of lipid per-oxidation in control and treated samples were measured by the formation of thiobarbituric acid reactive substance (TBARS). Briefly, 2 ml of TBA (0.7%) were added and sample heated at 100°C for 30 min. After cooling, the solutions were centrifuged at 1500 x g for 10 min. The absorbance of the pink supernatant was measured at 532 nm. To prevent oxidation during the assay, 67µM Butylated hydroxytoluene (BHT) was added at the beginning of the assay. MDA concentration was calculated using 1,1',3,3'-tetramethoxypropane as standard and was expressed as mg/dL of MDA.

2.6 Trace Elements Determination

Aliquots of serum samples (150 µL) were mixed with concentrated nitric acid (600 µL) and 30%

hydrogen peroxide (400 µL) into a pre-cleaned 15 mL polypropylene tube (Cen-Med). The tubes were loosely capped, centrifuged for 10 minutes at 4400 rpm. Then the sample was heated at 96°C on a heating block digester for 90 min [25].

The analysis of serum samples has been carried out using ICP-OES at Nuclear research Center, Tripoli, Libya. ICP-OES (model: VISTA-MPX, CCD simultaneous ICPOES, VARIAN, nebulizer type: glass concentric with pressure of 200 kPa, Perkin Elmer, USA) was operated under suitable conditions including choosing the suitable wavelength for each element (Mn 257 nm, Cr 267 nm, Cu 324 nm, Zn 213 nm, Co 228 nm, Mg 280 nm, Ni 232 nm, V 290 nm and Se 196 nm) with plasma argon flow rate of 12 L/min, auxiliary argon flow rate of 1.5 L/min, nebulizer argon flow rate of 0.4 L/min, integration time 100 s, read delay 20 s, and peristaltic pump flow rate of 1 mL/min. The measurement of Seronorm™ Trace Elements Serum quality control samples as compared with Seronorm P TM P certified values.

2.7 Statistical Analysis

Computer package IBM SPSS (Statistical Package for Social Science) version 17.0 was used in statistical analysis. All data were expressed as the mean ± standard deviation and data obtained in the study were compared by two – tailed t-test for unpaired data. Association between all parameters was determined using multiple comparisons and Pearson's correlation coefficient (r). *P*-values less than 0.05 were considered significant.

3. RESULTS

Table 1 shows the distribution of study groups according to age. For subjects with type-2 diabetes, the mean of patient's age was 55.43 years (range, 30–70 years), Among the different age groups, there were 17 patients (23.6%) age < 50 years, 51 patients (70.8%) ages of 50-69 years, while 4 patients (5.6%) age ≥ 70 years. The mean non-diabetic subjects (control) age was 51.05 years (range, 30–72 years), Among the different age groups, there were 10 subjects (47.6%) ages of 30-40, 10 subjects (47.6%) ages of 50-69 years, and 4 subjects (4.8%) age ≥ 70 years.

The diabetic patients had slightly excess body weight compared to the control subjects The mean duration of diabetes was 8.13±4.86 years,

and 31.9% of diabetic patients and 40% of non-diabetic subjects were smokers (Table 2).

Table 1. Distribution of subjects according to their age

	Age groups (years)			n
	30-49	50-69	>70	
Control (n)	10	10	1	21
Diabetics (n)	17	51	4	72

Table 2. Description of physical characteristics for diabetic and non-diabetic (control) subjects

Parameters	ND	T2DM
n	21	72
BMI	27.80±6.13	28.28±4.46
Duration of diabetes (years)	-	8.13±4.86
Smoking	40%	31.9%

Values are expressed as mean ±SD for n subjects, ND: Non diabetic, BMI, Body mass index, T2DM, non-insulin dependent (Type-2) diabetes mellitus

The results showed a highly significant increase in serum FBS and HbA_{1c} in T2DM patients as compared to non-diabetic subjects ($P<0.001$) (Fig. 1).

Analysis of serum lipid profiles included measurements of parameters such as cholesterol, triglycerides, HDL-C, and LDL-C in both T2DM patients and non-diabetic subjects (Fig. 2). The obtained results showed no significant change in HDL-C between T2DM and

non-diabetic subjects. However, highly significant increase in serum concentrations of LDL-C, TG and total cholesterol was markedly observed in T2DM patients as compared to non-diabetic subjects ($P<0.001$).

As shown in (Fig. 3), serum TABRS concentration was significant higher in T2DM group (0.036 ± 0.069 mg/dl) than control group (0.027 ± 0.041 mg/dL) ($P<0.05$).

As shown in (Table 3), most of element concentrations in T2DM males sera were markedly lower as compared to the control group, except the level of Cu which was significantly higher in patients with type-2 diabetes than control subjects ($P<0.000$).

In comparison with control, the serum level of Mg, Zn and V were found to be significantly decreased ($P<0.001$) in diabetic patients; the mean values for non-diabetic subjects were 12.21, 1.17 and 0.017 mg/L respectively, whereas the mean values for T2DM patients were 6.16, 0.809 and 0.005 mg/L respectively.

In addition, the serum levels of Cr and Co were significantly diminished in T2DM patients as compared to control group ($P<0.05$). As illustrated from Table 3, the mean values of Ni, Mn and Se showed no significant difference between patients with T2DM and the control group ($P>0.05$). Also, the mean serum Cu level was noticeably elevated in T2DM group as compared to non-diabetic subjects ($P<0.001$); the mean value for control group was 0.862 mg/L while the mean for T2DM group was 1.24 mg/L.

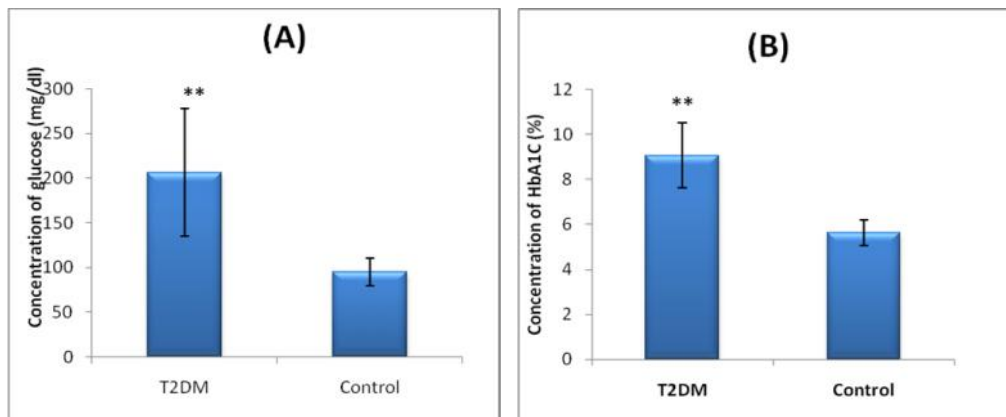


Fig. 1. Concentrations of FBS (A) and HbA_{1c} (B) in serum of male T2DM patients compared to non-diabetic subjects (male)

Data are mean ± SD; ** $P<0.001$ for T2DM compared with control group

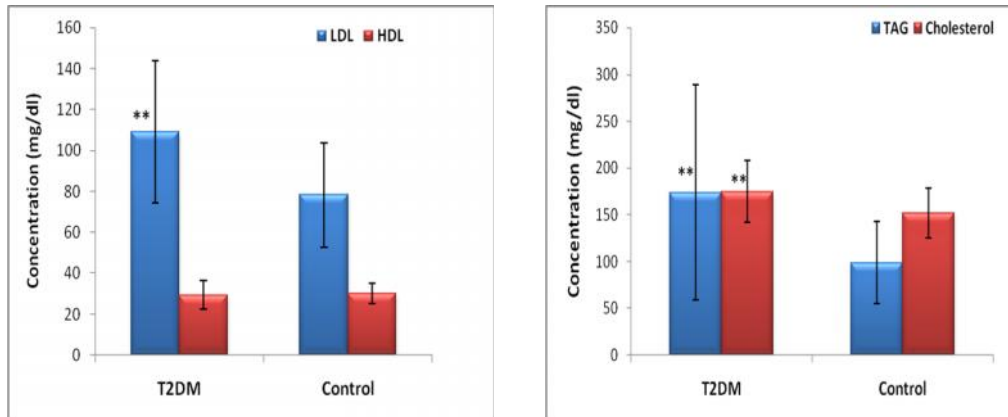


Fig. 2. Concentrations of LDL-C, HDL-C, TG and Cholesterol in T2DM male patients compared to non-diabetic subjects, respectively

Data are mean ± SD; **P<0.001 for T2DM compared with control group

Table 3. Serum level of trace elements and Mg in male diabetic patients and non-diabetic (Control) subjects (Mean±SE)

Element (mg/L)	Group		P Value
	T2DM	Non-diabetics	
Zn	0.809±0.329**	1.17±0.198	0.000
Cu	1.24±0.375**	0.862±0.303	0.000
Cr	0.061±0.070*	0.105±0.045	0.008
Ni	0.209±0.177 ^{NS}	0.177±0.160	0.427
Co	0.035±.034*	0.078±0.153	0.028
Mg	6.16±3.24**	12.21±2.59	0.000
Mn	0.069±0.084 ^{NS}	0.059±0.091	0.645
Se	0.173±0.331 ^{NS}	0.331±0.533	0.055
V	0.005±0.008**	0.016±0.018	0.000

* Significant at (P<0.05) between control and Diabetics subjects

** Highly Significant at (P<0.001) between Diabetics group and Non-diabetics group subjects

In order to examine possible association between trace elements levels and other biochemical parameters in diabetic patients, further statistical analyses (Pearson's correlation) were applied. Cr was inversely correlated with HbA1c (r=-0.25); Mg was inversely correlated with HbA1c and MDA (r=-0.34 and -.25, respectively) (Table 4); Ni showed a positive correlation with Cu and Cr (r= 0.332 and 0.360 respectively). In addition, positive correlation was found between Mg and Mn with Cu (r=0.262, 0.296, respectively) and V was correlated with Mg (r=0.250) (Table 5).

4. DISCUSSION

DM is a chronic disease that requires long-term medical attention to limit the development of its devastating complications and to manage them when they do occur [26]. There is accumulating

evidence that the metabolism of several trace elements is altered in diabetes mellitus and that these nutrients might have specific roles in the pathogenesis and progress of this disease [27, 1].

In the present study, fasting blood glucose level was significantly higher in diabetic patients than in healthy subjects.

Serum HbA_{1c} of the diabetic patients and control subjects are presented in (Fig. 1B), which was substantially higher in T2DM group in comparison with control group (P>0.001) indicating a poor or inadequate controlled diabetes in patients. Present study showed increase in HbA_{1c} which is associated with dyslipidemia, causing fatty liver and therefore increases liver enzyme activities as reported in previous study [28].

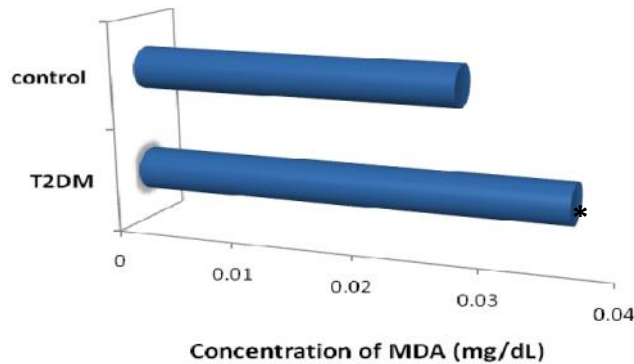


Fig. 3. Thiobarbituric acid test values obtained for T2DM patients and non- diabetic subjects
 Data are mean ± SD; *P<0.05 for T2DM compared with control group

Table 4. Pearson Correlation of trace elements and Mg with biochemical parameters among T2DM patients group

Element	FBS	HbA _{1c}	TG	Chol	LDL	HDL	MDA
Zn	-0.154	-0.061	0.021	-0.133	-0.131	-0.134	-0.068
Cu	-0.153	-0.034	-0.141	-0.104	-0.035	0.012	0.099
Cr	-0.034	-0.250*	0.094	-0.001	0.077	-0.058	0.011
Ni	-0.016	-0.201	-0.198	-0.071	-0.001	0.115	-0.010
Co	0.118	0.210	-0.076	0.028	0.204	-0.005	-0.009
Mg	-0.178	-0.340**	0.054	-0.102	-0.016	-0.039	-0.249*
Mn	0.071	0.072	0.028	0.001	-0.103	0.016	-0.038
Se	0.097	-0.095	0.215	0.040	0.060	-0.137	0.169
V	0.086	-0.110	0.007	-0.196	-0.007	-0.199	-0.108

*Statistical significance: P<0.05, FBS: Fasting blood glucose, HbA_{1c}: Glycosylated hemoglobin ,HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol, MDA: Malondialdehyde

Table 5. Pearson Correlations of trace elements and Mg in study patients with type 2 diabetes mellitus and control subjects

Element	Zn	Cu	Cr	Ni	Co	Mg	Mn	Se	V
Zn	1								
Cu	0.168	1							
Cr	0.084	0.078	1						
Ni	0.035	0.332**	0.360**	1					
Co	-0.107	0.199	0.003	0.115	1				
Mg	0.020	0.262*	0.024	0.182	-0.054	1			
Mn	-0.026	0.296*	-0.024	0.182	0.057	-0.052	1		
Se	-0.089	0.091	0.150	-0.001	-0.047	-0.029	0.068	1	
V	-0.020	0.201	0.009	0.018	-0.104	0.250*	-0.167	-0.026	1

** Correlation is highly significant at the 0.001 level, * Correlation is significant at the 0.05 level

Patients with T2DM often exhibit an atherogenic lipid profile, which greatly increases their CVD risk compared with non-diabetic subjects. The lipid profile parameters in diabetic patients were estimated, among their levels of LDL and cholesterol in diabetic patients were markedly higher (P<0.001) in comparison with their levels in control group, (Fig. 2). A high level of lipid per-oxidation in type-2 and type 1 diabetic patients was early reported [29]. The increased levels of

TBARS in diabetic patients may be due to increase in oxidative stress as well as compositional changes in LDL, which lead to more exposure of fatty acids to oxygen free radicals that enhance a faster rate of lipid per-oxidation [30]. This was in parallel with present observation as well as previous findings which observed that oxidized LDL is thought to promote atherogenesis by increased levels of secondary lipid per-oxidation products [31]. In the current

study, the level of lipid per-oxidation was higher in the serum of subjects with T2DM as compared to control group (Fig. 3), which was in concurrence with other study [15]. It has been stated that intensive oxidative stress may accelerate the metabolic deterioration of β cells in T2DM [32]. Excessive lipid per-oxidation has been suggested to be a potential biochemical lesion associated with the development of diabetic angiopathy like retinopathy and nephropathy [33].

As regards to the status of trace elements as well as Mg obtained from this study showed that the levels of Se and Zn, Co, Mg, Mn, V and Cr were diminished in serum of T2DM (Table 3). The loss of these minerals might be attributed to impaired absorption and/or the excess excretion of these minerals in urine (glycosuria) of these patients, which may induce a deficiency of these metals in diabetic patients.

Diabetic condition results in increased glomerular filtration rate and it is suggested that such an alteration in renal function may contribute to the increased urinary losses of the trace elements. Increased excretion of some trace elements as Zn indicates an abnormality of production or breakdown of metalloenzymes or metal-enzymes complexes [3]. The results in this study indicate a significant decrease in serum Zn level of diabetic patients compared to the non-diabetic subjects (Table 3) which may be related to loss of Zn in urine [34]. This was in accordance with previous finding [35,36]. In addition, the present results indicated that serum zinc level in diabetic group had no significant correlation with either FBS or HbA_{1c}, which was in parallel with a previous study [37]. Also previous study has shown that Zn plays a role as an antioxidant that could protect insulin and cells from being attacked by free radicals [38]. Therefore, there is an association between many of diabetes complications and the decrease in intracellular Zn and Zn dependent antioxidant enzymes [39]. Hence, when fats are oxidized, they are believed to become more reactive and damaging to the vascular system [40]. These results are in agreement with previous studies, which showed that the levels of Zn and Mg were decreased in the majority of cases of T2DM [41,42].

Table 3 showed a significant statistical differences between diabetic group and non diabetic group regarding to Cu concentration (*P*-value= 0.000). The higher concentration of Cu in subjects with T2DM might be due to increase in

glycation, which will stimulate release of copper from copper rich compounds such as ceruloplasmin [15] and the impaired synthesis of this transport protein. Therefore, the results confirm that T2DM have impaired Cu metabolism [41].

In this concern, results agree with some studies and contradict with others as one study revealed that the copper value in the diabetic patients was insignificantly different from the control subjects [42], while in another study reported that copper concentration was significantly higher in T2DM patients than non-diabetic subjects [15]. However, the third study found that copper values of diabetics showed no significant difference compared with controls [43].

The present work shows that a positive significant correlation was found between Cu and Ni ($r=0.332$), Cu and Mg ($r=0.262$) as well as Cu and Mn ($r= 0.296$) in T2DM male group (Table 5). Earlier study showed a correlation between Cu and Mg in type-2 subjects [32]. Increasing Cu in T2DM subjects may enhance the lipid per-oxidation; especially the oxidation of LDL-C leading to increased TBARS in diabetic patients as reported in the present study (Fig. 3).

Diabetes mellitus has been suggested to be the most common metabolic disorder associated with magnesium deficiency [44]. The mean serum Mg level was higher in the control group than in the diabetic group, where the *P*-value were statistically significant ($P<0.001$) (Table 3). The present result is in agreement with previous studies in which Mg level was found to be decreased in patients with diabetes compared with control group [45].

In subjects with T2DM there was negative correlation between serum Mg level and HbA_{1c} ($r= -0.340$), while a positive correlation was found between Mg and V ($r= 0.250$), (Tables 4 and 5). Moreover, a negative correlation was obtained between Mg and MDA ($r= -0.249$) (Table 4). It has been found that serum Mg levels are correlated inversely with HbA_{1c} [9]. Loss of Mg in T2DM is often associated with both extracellular and intracellular Mg depletion [46]. Epidemiologic studies have found high incidence of hypomagnesaemia in subjects with T2DM, especially in those with poorly controlled glycemic control [46]. Moreover, Mg deficiency has been proposed as a factor in the pathogenesis of diabetes-related complications, including neuropathy [47].

The mean plasma Se content in T2DM diabetic patients was lower (0.146 ± 0.373 mg/L) than in control subjects (0.185 ± 0.215 mg/L) (Tables 3). The correlation between plasma decreased selenium level and metabolic risk factors for cardiovascular disease appears to be positive [48].

Mn is a cofactor of many enzymes including mitochondrial superoxide dismutase [26]. Mn-activated enzymes play important roles in the metabolism of carbohydrates, amino acids, and cholesterol [49]. There is evidence that Mn may be involved in the pathogenesis of diabetes [50]. Mn has been also shown to be important in insulin synthesis and secretion [26]. In our study, serum Mn level of diabetic patients was lower than the non diabetic subjects (Table 3). A positive correlation was found between Mn and Cu ($r = 0.262$) (Tables 4 and Table 5).

Diabetes has been shown to be associated with abnormalities in the metabolism of chromium and zinc, they play role in the progression of the ailment. Chromium is a cofactor in the action of insulin [47,22]. There were significant differences between serum Cr in male diabetic in comparison with control subjects ($P = 0.008$) (Table 3). Also, in diabetic group there were an inverse correlation of [Cr] in serum and HbA_{1c} , ($r = -0.250$) (Table 4).

In diabetic group, the serum V contents were remarkably diminished as compared to controls (Tables 3). In addition, a positive correlation between V and Mg (Table 5) was also observed. Current results are in agreement with one study which proposed that a decrease in V level may be resulting from the glomerular hyperfiltration in diabetes [51], contrary to other results which reported an increase in V concentration in T2DM subjects [52]. Despite the little impact of V on human metabolism, it is believed that V supplements could enhance hepatic and muscle insulin sensitivity, reduces levels of fasting blood sugar [53] and therefore it is well recommended as treatment for type-2 diabetes [54]. The level of Ni was higher in serum of T2DM subjects than control groups (Table 3). That was in agreement with previous study [55].

5. CONCLUSION

This study shows that the decrease in trace elements and increasing in lipid per-oxidation products appear to be an additional risk factor in the pathogenesis of type-2 diabetes mellitus and

its complications. In addition, trace elements could be used as markers to evaluate the glycemic control and the lipid status of diabetic patients. Although some of the present findings show good agreement with previous findings of other researchers in the same concern, more work is required to clarify the effect of trace elements metabolism on diabetes and *vice versa*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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