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Assessment of Plasma Copper, Zinc, and Glycated Hemoglobin among Sudanese Patients with Type 2 Diabetes Mellitus in Khartoum State

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Abstract: Diabetes mellitus (DM) describes a metabolic disorder of multiple etiologies. It is characterized by chronic hyperglycemia associated with disturbances of carbohydrate, fat and protein metabolism, which result from defects in insulin secretion, insulin action. The objective of this study is to assess some of the biochemical parameters related to diabetic patients. This is a descriptive, analytic, cross-sectional, and hospital based study. The scope of this study is to assess the plasma trace elements, glycated haemoglobin among Sudanese patients with types 2 diabetes mellitus in comparison with healthy Sudanese volunteers. This study took place during the period between September 2013 to June 2016 in Khartoum state, where is the capital. Its, population is about five million inhabitants. Blood parameters were measured using spectrophotometric methods. The (mean ±SD) of glucose, HbA1, zinc and copper in healthy subjects respectively were (93.5±10.5, 4.8±0.8, 103.7±82.5,103.8±66.5). And the (mean ±SD) of glucose, HbA1, zinc and copper in patients respectively were (196.6±75.7, 7.9±2.1, 132.0±89.6, 107.0±68.6). There was significantly increased of zinc and HbA1 and also increased in means of copper in patients compared to control groups.
Keywords: Trace element, diabetic patients, Sudanese.

INTRODUCTION

Diabetes mellitus (DM) describes a metabolic disorder of multiple etiologies. which is characterized by chronic hyperglycemia, with disturbances of carbohydrate, fat and protein metabolism, which result from defects in insulin secretion, insulin action, or both [1]. Non-insulin-dependent diabetes mellitus is one of the most widely spread and severe disorder currently. The number of patients suffering from diabetes mellitus was reported to be over 381.8 million people worldwide [2]. There is accumulating evidence that the metabolism of several trace elements are altered in DM and that these nutrients might have specific roles in the pathogenesis and progression of this disease [3]. A relationship between trace and macro elements with diabetes has been observed in many research studies. The proposed mechanism of trace elements enhancing insulin action includes activation of insulin receptor sites and serving as co-factors or components for enzyme systems involved in glucose metabolism [4]. Alteration in the metabolism of trace elements like copper is associated with DM [5]. Trace elements are accepted as essential for optimum health, because of

their diverse metabolic characteristic and functions [6]. 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 [5, 7]. Copper acts as a pro oxidant and may participate in metal catalyzed formation of free radicals [5]. The increased production of free radicals is likely to be associated with development of type 2 DM [5, 8]. And on the other hand some of these nutrients can directly modulate glucose homeostasis [9, 10]. Deficiencies of certain minerals such as Zn have been shown to predispose a person to glucose intolerance and to promote the development of diabetic complications [11]. It was reported that Zn is 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 [12]. 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 [13].

The objective of this study was to assess plasma trace elements, glycated haemoglobin among Sudanese patients with types 2 diabetes mellitus in comparison with healthy Sudanese volunteers.

MATERIAL AND METHODS
Study population

This is a descriptive, analytic, case and contol, and hospital based study. This study took place during the period between September 2013 to June 2016 in Khartoum state, one of Sudan's eighteen states, and is situated at the center where, Khartoum, the capital. This state constitute region, which is one of the most crowding area in Sudan, and most of the medical and education and other social services are centralized in the capital. The study samples comprised 250 Sudanese patients with type 2diabetes mellitus; in contrast, 150 healthy volunteers (age and sex matched) are involved as control group. Sudanese patients with type 2 diabetes mellitus are selected from the main cities, Khartoum (Jabir Abulizz centre for diabetes patient), Khartoum North-Bahre (Al-Amal national hospital).

These subject were chosen from male and female (patients and control group) after they were diagnosed and be cheeked by a physician in each clinical.

Inclusion criteria

Test group: Sudanese patients with type 2 diabetes mellitus.

Control group: healthy subjects. (Non-diabetics).

Exclusion criteria

Patients with type 1 diabetes mellitus, renal failure, liver disease, anemia, and thyroid disease were excluded from this study.

Ethical Consideration

Permission of the study was obtained from Medical director of both Al-Amal national hospital and Jabir AbulizzCentre in Khartoum state. The aims and the benefits of the study were explained to the all participant with assurance on confidentiality, also an informed consent was obtained from all participants. An Interview with the patients were done in order to get clinical data and also to fill the questionnaire by the patient.

Blood samples collection

After informed consent, Blood samples (7 ml) were collected from fasting subjects of both the study group and control by standard procedures. Collected blood was drawn in three containers (heparin, fluoride oxalate and EDTA). The blood in the containers was gently mixed with the anticoagulant to obtain plasma and whole blood consecutively. Heparin container was centrifuged at 10000 rpm at -4°C using cold centrifuge. Hemolyzed and lipemic samples were rejected and excluded from the study. The whole blood samples were used immediately after collection for testing glycocylated hemoglobin, plasma samples were preserved at -20°C prior to processing. Heparinized plasmas were used for testing copper, zinc and while plasma from fluoride oxalate for testing fasting glucose. Fasting blood glucose was determined by using enzymatic method (glucose oxidase/ peroxidase) by commercial kit (Biosystem S.A Costa Brava 30, Barcelona – Spain). Levels of HbAIc were determined by method based on boronate affinity chromatography by using NYCOCARD READER II-AXIS-SHIELD PoCAs NO-0504 (Oslo, Norway). Zinc and copper were determined by using diagnostic reagent by commercial kit (DialabA-2351 Wr. Neudorf – Austria by Autoanlyzer T60.UV-VIS spectrophotometer - England.).

Quality Control

The precision and accuracy of all methods use in this study were checked at each batch using commercially prepared control sera.

Data Collection

Data were collected from both the questionnaire and the blood samples.

Statistical analysis

Statistical Package for Social Science SPSS (version 13) computer software was used for data analysis. The means and standard deviations of the plasma levels fasting plasma glucose and blood glyclated hemoglobin (HbA1c), copper and Zinc were calculated and T-test (independent T samples) was used for comparison (significant level was set at $P \leq 0.05$).

Linear regression analysis was used to assess the correlation between the HbA1c, duration of diabetes, and BMI and the plasma levels of fasting plasma glucose, copper, zinc and blood glyclated hemoglobin (HbA1c). The results presented in form of tables and figures.

RESULTS

**Table 1: Baseline characteristics of the respondents**

Variables	Control group (non-diabetics) (n=150)	Test group (diabetics) (n=250)	P-value
Age (Years) (Min-Max)	**49.1±13** **(36.0-62.0)**	**51.2±13** **(38.0-64.0)**	**0.1**
Weight(Years) (Min-Max)	**64.8±10.4** **(50.0-109.0)**	**66.3±8.8** **(49.0-89.0)**	**0.17***
Height (Years) (Min-Max)	**167.5±6.4** **(148.0-190.0)**	**163±8.7** **(142.0-192.0)**	**0.001***
BMI (Years) (Min-Max)	**23.1±3.3** **(17.7-36.2)**	**49.9±3.1** **(24.0-36.0)**	**0.001***

- The table shows the mean ±SD.
- t-test was used for comparison.
- *P value ≤ 0.05 is considered significant.

Table 2: Comparison of the means of Blood Parameters between diabetics and none diabetics

| Variables	None-diabetics (n=100)	Diabetics (n=300)	P value
Copper (µg/dl) (Max-Min)	**103.8±66.5** **(348.0-6.2)**	**107.0±68.0** **(551.0-4.0)**	**0.64***
Zinc (µg/dl) (Max-Min)	**103.7±82.5** **(865.7-10.9)**	**132.0±89.6** **(766.2-11.4)**	**0.002***
HbA$_1$c% (Max-Min)	**4.8±0.8** **(6.8-2.6)**	**7.9±2.1** **(12.9-3.1)**	**0.003***
Glucose (mg/dl) (Max-Min)	**93.5±10.5** **(120-69.0)**	**196.6±75.7** **(432.0-65.0)**	**0.006***

* Significant differences in all blood parameters (except copper not significant P > 0.05) between control and test group (P value < 0.05).
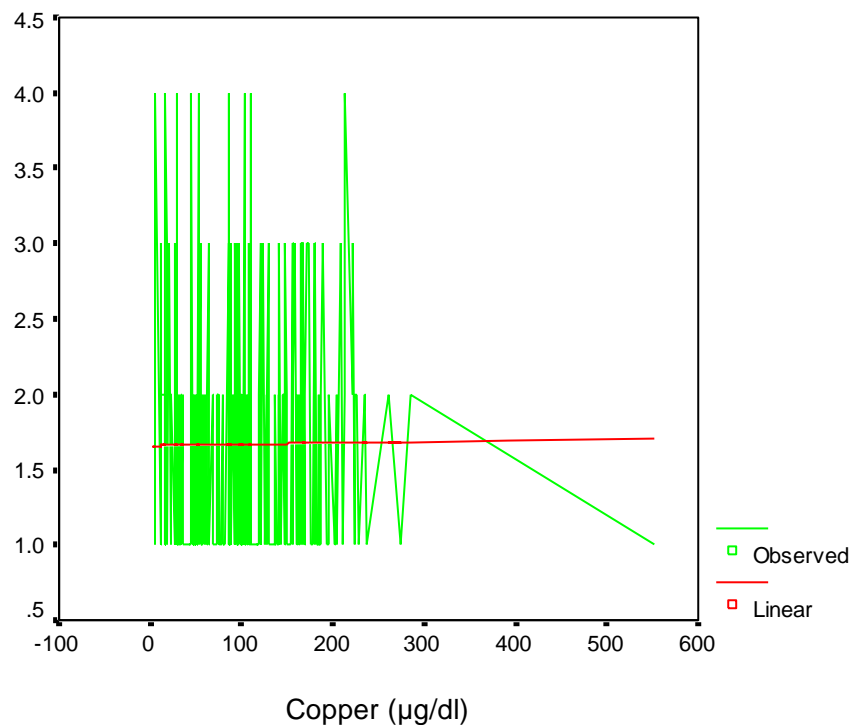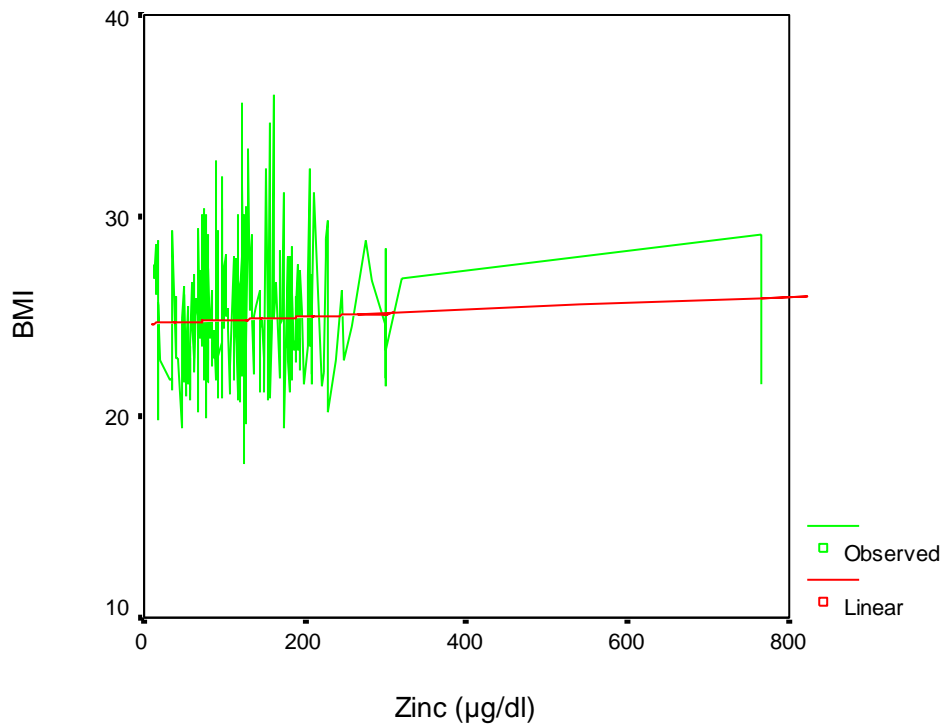

Fig-1: The relationship of the serum copper (µg/dl) of and duration of the disease
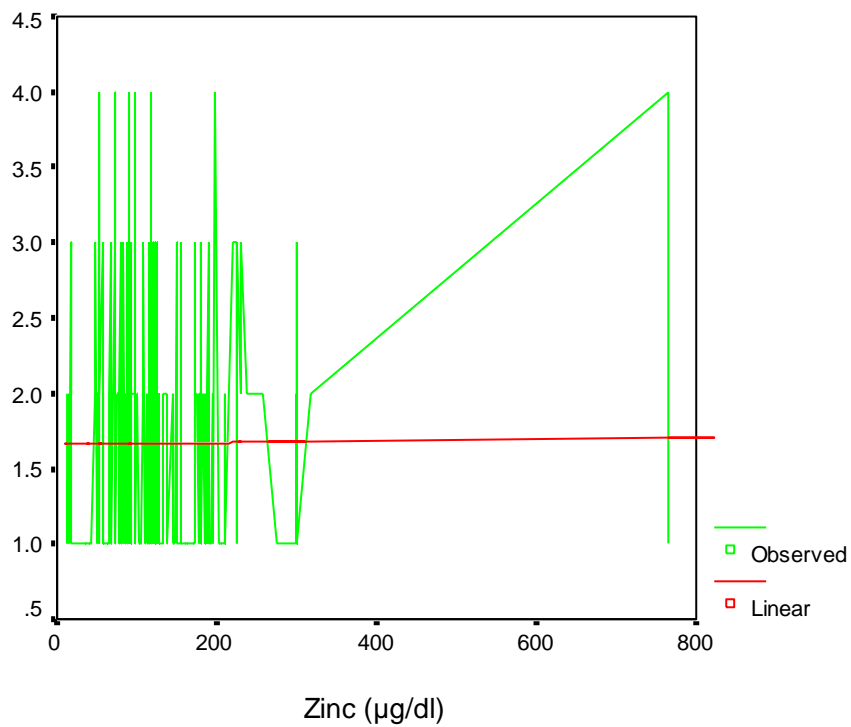R = 0.06 P value = 0.07 (negative correlation)

BMI
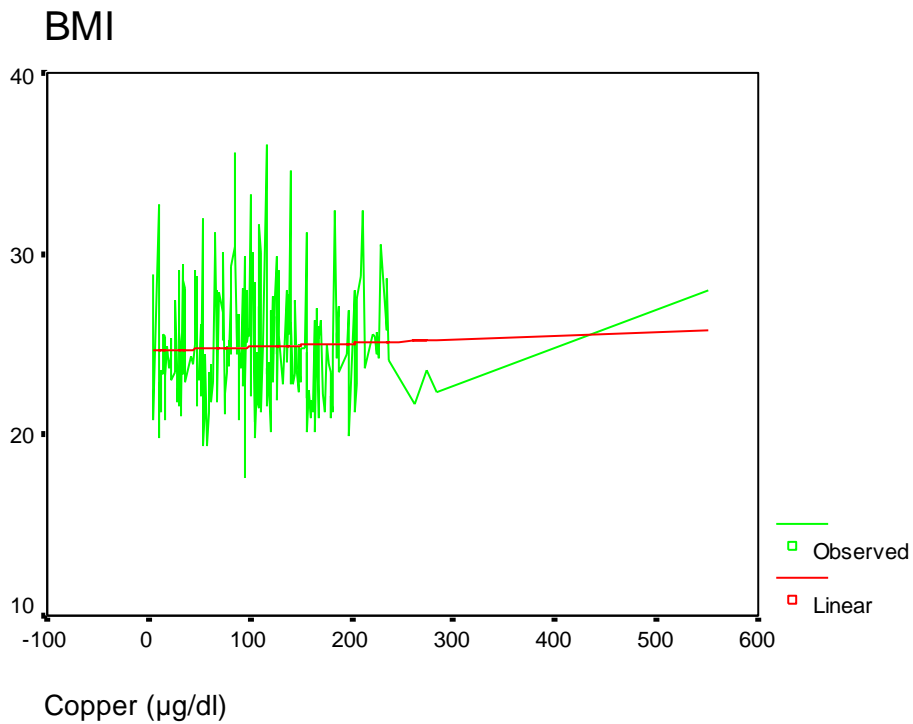
Fig-2: The relationship of the serum copper (µg/dl) of and BMI
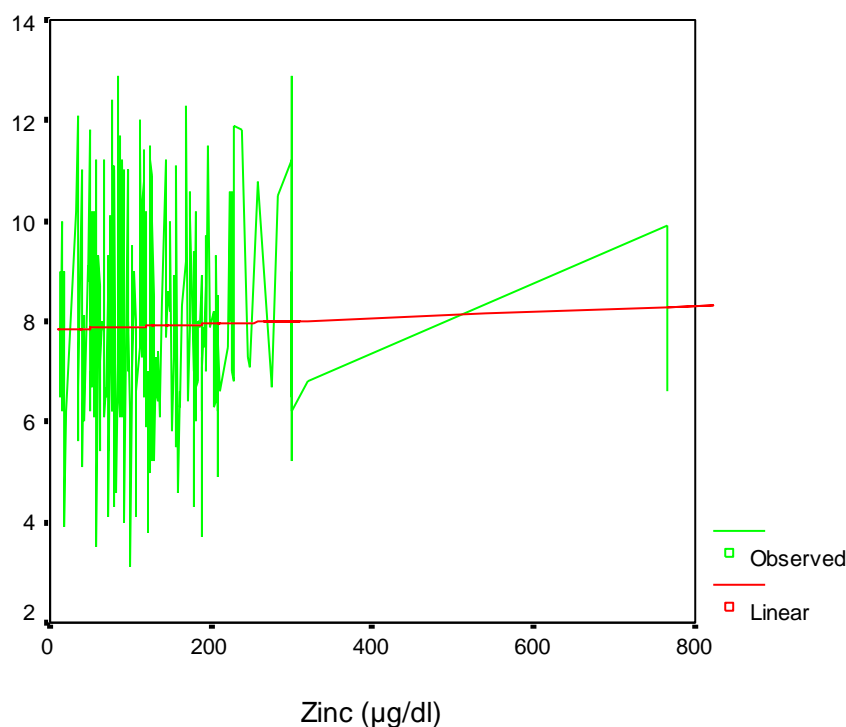R = 0.007 P value = 0.90 (negative correlation)
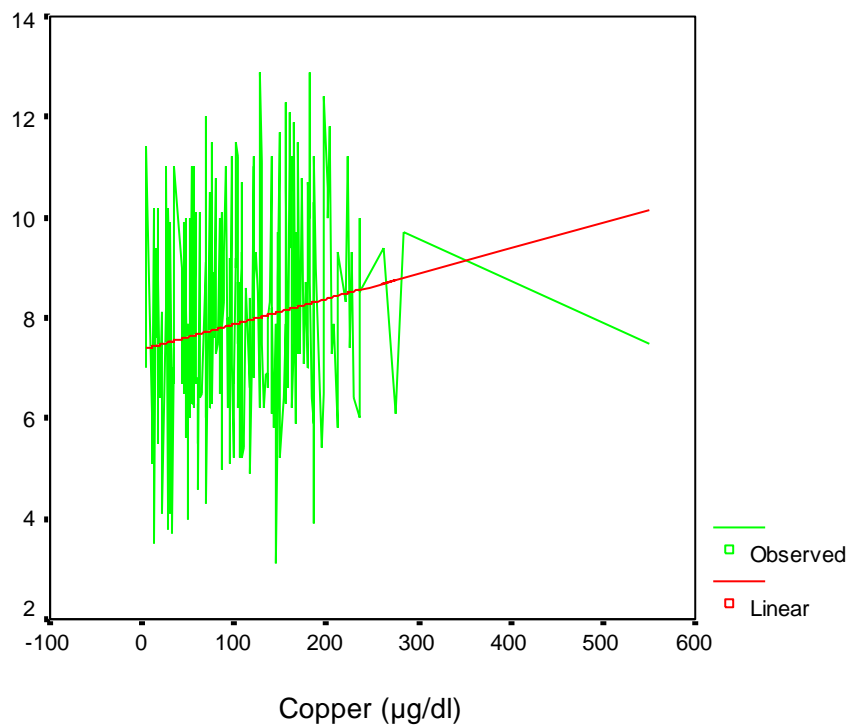
Fig-3: The relationship of the serum copper (µg/dl) of and HbA$_{1c}$%
R = 0.17 P value = 0.01 (positive correlation)

Fig-4: The relationship of the serum zinc (µg/dl) of and BMI
R = 0.05 P value = 0.43

Fig-5: The relationship of the serum zinc (µg/dl) of and duration of the disease
R = 0.005 P value = 0.94 (negative correlation)

Fig-6: The relationship of the serum zinc (µg/dl) of and HbA$_{1c}$%
R = 0.025 P value = 0.67

Table 3: gender association with type 2 diabetes mellitus in Sudanese patients

	Gender	N	Mean	Std. Deviation	P value
Glucose (mg/dl)	Male	99	207.1	86.4	0.07*
	Female	151	189.7	67.2	
HbA$_{1c}$%	Male	99	7.8	2.2	0.67*
	Female	151	8.0	2.0	

* No Significant differences in blood glucose and HbA$_{1c}$% between diabetic males and females (P value > 0.05).

DISCUSSION

Diabetes is one of the fastest-growing health problems in the world (especially type 2), which is reaching epidemic proportion in some regions, as consequence of life-style, lack of exercise, unhealthy diet, obesity and overweight. In 2013, 382 million people had diabetes; this number is expected to rise to 592 million by 2035 [14]. During the past 20 years, major socio demographic changes have occurred in Sudan, and changes in physical activity and dietary patterns have promoted the development of non-communicable disease, such as diabetes mellitus. The micro vascular and naturopathic complications of diabetes mellitus are a major clinical and public health problem in Sudan.

In these study 250 Sudanese patients with Type 2 diabetes mellitus as test group and 150 apparently healthy subjects (non-diabetics) as control group were participated.

The current study was found that the weight and BMI were significantly higher among the test than apparently healthy control group. Body mass index of diabetic subjects found to be higher than that of non-diabetics. The mean BMI of control group was found to be 23.1 kg/m^2, which is slightly above the normal range of recommended BMI by WHO for healthy individuals, on the other hand the mean of BMI of test group was found to be 24.9 kg/m^2, indicating overweight and this agree with the study done by Helen, *et al.* [15]. Changes in BMI and weight before and after the development of type 2 diabetes. Similar studies had been reported that there is strong interrelation between BMI and type 2 diabetes Mellitus which states that increase in BMI predisposes to type 2 diabetes mellitus [16]. Also, another study done in USA found that obesity and overweight strongly correlated with diabetes mellitus among American subjects [17].

The current study revealed the copper concentration in diabetic patients was slightly higher than in normal individuals, but no significant relationship was found, 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 [18]. The higher Copper concentration in diabetic patients found in this study was consistent with the studies of Kazi*et al*. [19]. Similar results were also observed in the study of Zargar*et al*. [20]. who confirmed the relationship between diabetes and essential metals. Craft and Faillain their study suggested that diabetic patients probably absorb twice as much dietary Cu as normal individuals, and may have a narrower safe range of intakes. Cu is a metal that can potentially act as a pro-oxidant and cause oxidative formation of free radicals and destructive lipid peroxidative damage [21]. Copper has a particular role in cytochrome oxidase function at the terminal end of the mitochondrial electron transport chain. Activity deficiency in cytochrome oxidase function due to Cu deficit may contribute to distortion of mitochondria, particularly in metabolically active tissues such as pancreatic acinar cells, enterocytes and hepatocytes [20].

In this study among test group highly significant difference between the means of plasma copper of the hypertensive test group and the normotensive test group, this agree with study done by Ghayour-Mobarhan *et al*. [22] who reported that serum copper is higher in hypertensives. However, de la Sierra *et al*. reported that there is no correlation between the degree of endothelial dysfunction and serum copper or zinc levels [23]. In this study among test group significant difference between plasma copper of the control diabetic patients and the uncontrolled was found. Also shows a significant difference between the means of plasma copper of the test group on oral hypoglycemic drugs and those who did not. On the other hand, no significant weak correlation between duration of diabetes and plasma copper of the test group and significant moderate correlation between plasma copper and BMI and HbA1c was reported.

The present study shows highly significant difference between the means of plasma zinc of the test group and the control group. It is worth mentioning that despite the finding in many studies that zinc is decreased [24], also the results of this study agree with study done by D'Ocon *et al*, [25] that shown increased zinc in diabetes mellitus. Mateo *et al* [26] and Osman *et al*, [27] also observed similar results The increase in zinc levels in diabetics could be explained by the finding that oxidative stress in diabetics could lead to destruction of ß-cells, therefore to 49 the release of high amounts of zinc from the cells into blood stream, therefore increase in zinc levels in serum occurs [28], the increase of plasma zinc can also reflect a deficient storage or a chronic hyper secretion of insulin in hyperglycemic patients [26].

In current study among test group highly significant difference between the means of plasma Zinc of the hypertensive test group and the normotensive test group was found, this agree with study done by Taneja *et al*, [29] which reported increased serum zinc in hypertensives. Also there is highly significant difference between the means of plasma Zinc of the test group on diet control and those do not on diet control and no significant difference between the means of plasma Zinc of the test group on oral hypoglvemic drugs and those who were not. On the other hand, significant positive correlation between serum Zinc and BMI and duration of disease and insignificant weak positive correlation with HbA1c was reported.

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