Determination the Value of Chromium, Zinc, Phosphorus and Manganese in Patients with Type 2 Diabetes Mellitus by Different Techniques

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Abstract

Introduction: Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Insulin action was reported to be potentiated by some trace elements like chromium, zinc, manganese and phosphorus. Proposed mechanisms of enhancement of insulin action by trace elements include activation of insulin receptor sites, serving as cofactors or components for enzyme systems which are involved in glucose metabolism, increasing insulin sensitivity and acting as antioxidants for preventing tissue peroxidation. Aim: Estimation levels of serum zinc, phosphorus; chromium and manganese in diabetes mellitus type 2 patients and compare them with control group.Evaluation the impact of these levels on the severity of the disease, and determine the correlation between them.Comparsion of two different methods in determination of serum trace elements .Materials and methods: This study was conducted on 180 subjects attending Al- Suwayrah hospital between Octobers to December 2021. Subjects had age range within 35-65 years. This study is case-control study with sample size (180), include (90) T2DM patients (56 femal and 34 male) with normal renal function and (90) apparently healthy control group. Serum glucose, serum urea , serum creatinine , serum Zn and serum phosphate were determine by spectrophotometric to study groups (diabetic and control).Serum Zn,Ch,Mn were determine by Flameless Atomic absorption spectrophotometer method by the technique of Graphite Furnace (GFAAS) to study groups. Results: In this study, the mean differences of age and body mass index according to study group including (diabetic patients and control group).There were significant differences between means of age and body mass index according to study group In this study, Serum zinc measured by two methods spectrophotometric and atomic absorption. In two methods there were significant differences between means of Zinc according to study group P-value <0.001 (P value ≤ 0.05 was significant). There were significant differences between means of manganese and chromium (Atomic absorption) according to study group. There were no significant differences between means of Phosphate (spectrophotometric) according to study group. Conclusion: Larger link between BMI and the onset of diabetes.Low serum levels zinc,mangnese and chromium in patients with type 2 diabetes mellitus. As early in the development of DMT2 plasma phosphate levels may be normal or even low, these deregulations may be difficult to distinguish.

Keywords: Diabetes mellitus, Type 2, Oxidative stress, Zn, Ch, Mn, phosphorus, Superoxide dismutase, Metabolism

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone [1]. Chronic hyperglycemia statue in diabetes favors the manifestation of oxidative stress due to high production of reactive oxygen species and/or a decrease of the antioxidant defense system activity linked to lipid peroxidation and oxidative cellular injury themselves resulting in damages in the metabolism of lipids, proteins and DNA and from changes in cells functions [2]. Zinc protecting biological structures from damage by free radicals may be due to several factors: an adequate level and maintenance of metallothioneins (MTs), an essential component of superoxide dismutase (SOD), a protective agent for thiols (RSH), thus preventing the interaction between chemical groups with iron to form free radicals [3]. Metabolic disturbances including changes in phosphate can explain why dyslipidemia, hyperglycemia and hyperuricemia, which are related to obesity, impact the progression to type 2 DM. It has been reported that low serum levels of P are associated with increased insulin resistance in the healthy population. Moreover, a previous experimental study using rats suggested that phosphate depletion results in low insulin secretion by pancreatic beta cells, due to high intracellular calcium and inhibition of adenosine triphosphatase production [4]. Chromium might play a role in carbohydrate, lipid, and protein metabolism by potentiating insulin action [5, 6]. Although the

doi.org/10.31838/hiv22.02.344 Received: 27.05.22, Revised: 28.05.22, Accepted: 13.08.22

precise mechanism for this activity has not been identified, scientists have proposed that chromium binds to an oligopeptide to form chromodulin, a low-molecular-weight, chromium-binding substance that binds to and activates the insulin receptor to promote insulin action. Manganese is a cofactor for many enzymes, including manganese superoxide dismutase, arginase, and pyruvate carboxylase. Through the action of these enzymes, manganese is involved in amino acid, cholesterol, glucose, and carbohydrate metabolism; reactive oxygen species scavenging [7].

2. Materials and Methods

This study was conducted on 180 subjects attending Al- Suwayrah hospital between Octobers to December 2021. Subjects had age range within 35-65 years. This study is case-control study with sample size (180), include (90) T2DM patients (56 femal and 34 male) with normal renal function and (90) apparently healthy control group, sample size was calculated by sample size equation with the aim of the community health department in the college. .Exclusion criteria were type 1 diabetic patients, Patients with kidney diseases, Patients with pissed disease, pregnant women and Patients with cardiovascular disease. Body mass index is calculated by dividing weight (Kg) by height square (m^2) for all subjects.

Three to five milliliters of blood were obtained from diabetic patients and control, then collected in tube without anticoagulants and were left for 15 minutsat room temperature to clot. After that, the blood samples were centrifuged for 10 minutes.Then the sera were aspirated and stored at (-20 °C) until time of use.

Serum glucose, serum urea , serum creatinine , serum Zn and serum phosphate were determine by spectrophotometric to study groups (diabetic and control).Serum Zn,Ch,Mn were determine by Flameless Atomic absorption spectrophotometer method by the technique of Graphite Furnace (GFAAS) to study groups.

Ethical approval: The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number xxxx (including the number and the date in DD/MM/YYYY) to get this approval.

3. Results

This study enrolled, ninety patients suffered from diabetes meletus type 2(case group), 34 (37.8%) were males 56 (62.2%) were females. The control group was ninety adults, 34 were males and 56 were femals with mean age and body mass index, as shown in table (3.1).And figure (3.1) which show distribution of diabetic patients according to study variables including (age, gender and body mass index). Mean age of patients was (54.51 ± 6.69) years, minimum age was 40 years and maximum age was 69 years. Majority of patients (62.2%) was female and majority of patients (73.3%) were obese.

Table (3.1): The Distribution of patients according to study variables (N=90)		
Study variables		
Age (years)	(54.51 ± 6.69)	(40.0 - 69.0)
Gender		
Male	34	37.8%
Female	56	62.2%
Total	90	100.0%
BMI (kg/m2)		
Normal (18.5-24.9)	0	0.0%
Overweight (25-29.9)	24	26.7%
Obese (≥ 30)	66	73.3%
Total	90	100.0%
Mean body mass index was (32.92 ± 4.26)		

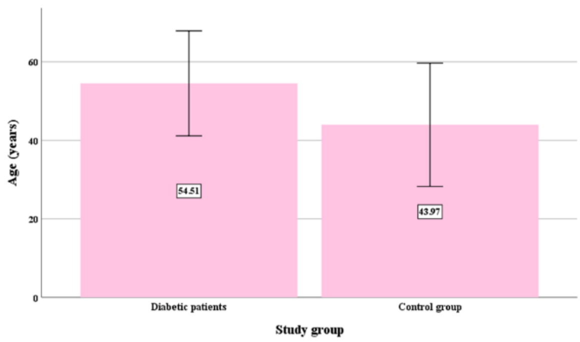

Figure (3.1): The mean differences of age according to study group (P<0.001)*

In this study ,the mean differences of age and body mass index according to study group including (diabetic patients and control group).There were significant differences between means of age and body mass index according to study group, as shown in table (3.2)and figure (3.2).

Table (3.2): The mean differences of age and body mass index according to study group					
Study variables	Study group	N	Mean±SD	t-test	P-value
Age (years)	Diabetic patients	90	54.51±6.69	9.694	<0.001*
	Control group	90	43.97±7.85		
BMI (Kg/m2)	Diabetic patients	90	32.92±4.26	7.366	<0.001*
	Control group	90	29.05±2.56		
*P value ≤ 0.05 was significant					

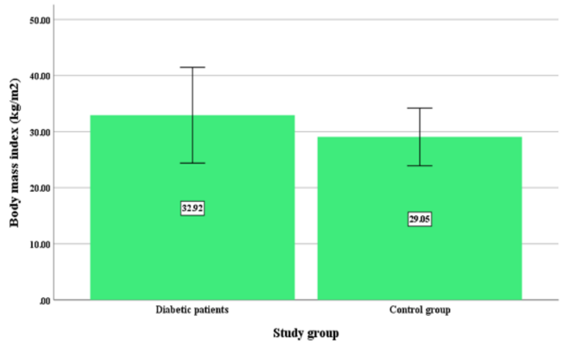

Figure (3.2): The mean differences of BMI according to study group (P<0.001)*

In this study, it measured blood urea, creatinine and fasting blood sugar (spectrophotometric) to study group including (diabetic patients and control group).There were significant differences between means of blood urea , serum creatinine and FBS (spectrophotometric) according to study group as shown in table (3.3)and figures (3.3),(3.4),(3.5).

Table (3.3): The mean differences of blood urea, serum creatinine and FBS (spectrophotometric) according to study group

Study variables	Study group	N	Mean ± SD	t-test	P-value
Blood urea/ sp (mg/dl)	Diabetic patients	90	29.36 ± 5.06	2.096	0.038*
	Control group	90	27.76 ± 5.18		
Serum creatinine / sp (mg/dl)	Diabetic patients	90	0.80 ± 0.17	2.755	0.006*
	Control group	90	0.73 ± 0.16		
FBS/ sp (mg/dl)	Diabetic patients	90	214.89 ± 78.33	15.391	<0.001*
	Control group	90	87.31 ± 6.87		

*P value ≤ 0.05 was significant

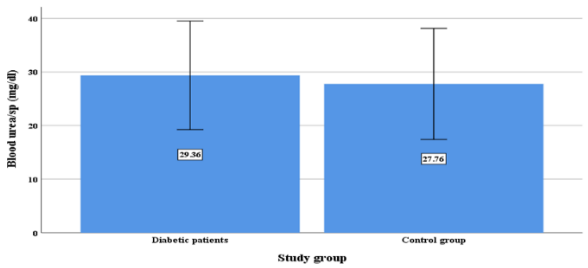

Figure (3.3): The mean differences of blood urea / sp according to study group (P= 0.038)*

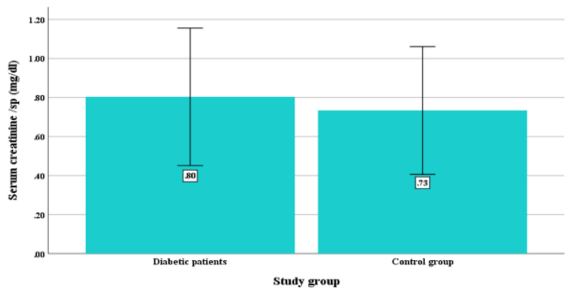

Figure (3.4): The mean differences of serum creatinine / sp according to study group (P=0.006)*

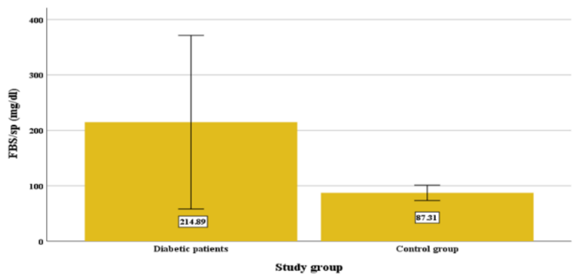

Figure (3.5): The mean differences of FBS / sp according to study group (P<0.001)*

In this study, Serum zinc measured by two methods spectrophotometric and atomic absorption. In two methods there were significant differences between means of Zinc according to study group P-value <0.001 (P value ≤ 0.05 was significant), as shown in tables (3.4), (3.5) and figures (3.6), (3.7).

Table (3.4): The mean differences of Zinc (spectrophotometric) according to study group

Study variables	Study group	N	Mean±SD	t-test	P-value
Zinc / sp (µg/dl)	Diabetic patients	90	105.20±5.82	-5.313	<0.001*
	Control group	90	110.30±6.99		

*P value ≤ 0.05 was significant

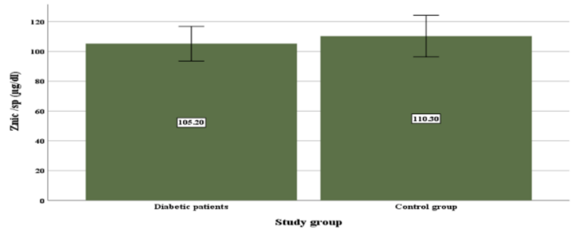

Figure (3.6): The mean differences of Zinc / sp according to study group (P=<0.001)*

Table (3.5): The mean differences of Zinc (Atomic absorption) according to study group

Study variables	Study group	N	Mean±SD	t-test	P-value
Zinc/atom (µg/dl)	Diabetic patients	90	53.79±5.48	-40.182	<0.001*
	Control group	90	80.64±3.17		

*P value ≤ 0.05 was significant

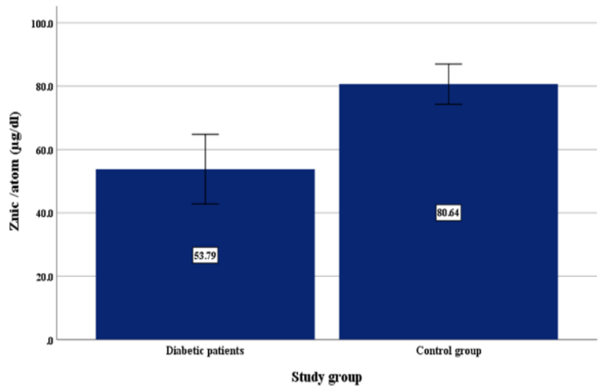

Figure (3.7): The mean differences of Zinc / atom according to study group (P=<0.001)*

In this study, the mean differences of Zinc (µg/dl) measured by two technique include (Spectrophotometric and Atomic absorption) among diabetic patients. There were significant differences between means of Zinc between these two techniques among diabetic patients, as shown in tables (3.6) and figure (3.8).

Table (3.6): The mean differences of Zinc according to technique of measurement among diabetic patients (N=90)

Study variables	Study group	N	Mean ± SD	Paired t-test	P-value
Zinc (µg/dl)	Spectrophotometric	90	105.20 ± 5.82	59.66	<0.001*
	Atomic absorption	90	53.79 ± 5.48		

*P value ≤ 0.05 was significant

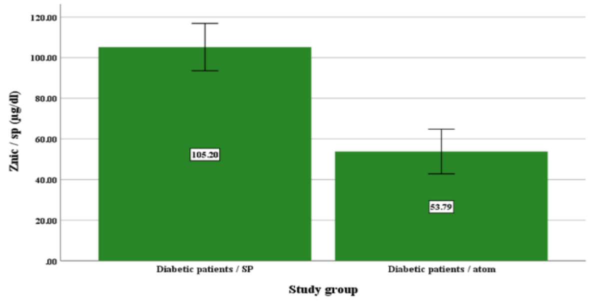

Figure (3.8): The mean differences of Zinc according to technique of measurement among diabetic patients (N=90, P<0.001)*

In this study, the mean differences of Zinc (µg/dl) measured by two technique include (spectrophotometric and atomic absorption) among control group. There were significant differences between means of Zinc between these two techniques among control group, as shown in table (3.7) and figure (3.9).

Table (3.7): The mean differences of Zinc according to technique of measurement among control group (N=90)					
Study variables	Study group	N	Mean ± SD	Paired t-test	P-value
Zinc (µg/dl)	Spectrophotometric	90	110.30 ± 6.99	37.501	<0.001*
	Atomic absorption	90	80.64 ± 3.17		
*P value ≤ 0.05 was significant					

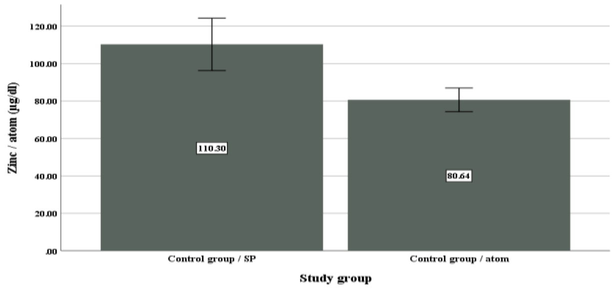

Figure (3.9): The mean differences of Zinc according to technique of measurement among control group (N=90, P<0.001)*

The ROC curve for the sensitivity and specificity of Zinc / Sp (µg/dl) for diagnosis of diabetes mellitus, (Cut-off point was ≤ 109.50 (µg/dl)) , AUC=0.71, P <0.001*, 95% CI (0.634-0.787), the sensitivity was 80.0%, the specificity was 61.1%, positive predictive value was 67.28%, negative predictive value was 75.34% and overall accuracy was 70.55%, as shown in figure (3.10).

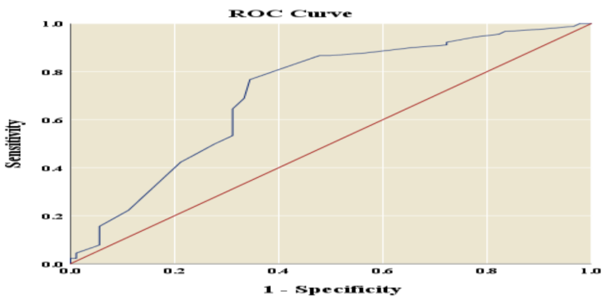

Figure (3.10): ROC curve for the sensitivity and specificity of Zinc / Sp (µg/dl) for diagnosis of diabetes mellitus

The ROC curve for the sensitivity and specificity of Zinc / atom (µg/dl) for diagnosis of diabetes mellitus, (Cut-off point was ≤ 75.40 (µg/dl)) , AUC=1.00, P <0.001*, 95% CI (1.000-1.000), the sensitivity was 100.0%, the specificity was 100.0%, positive predictive value was 100.0%, negative predictive value was 100.0% and overall accuracy was 100.0%, as shown in figure (3.11).

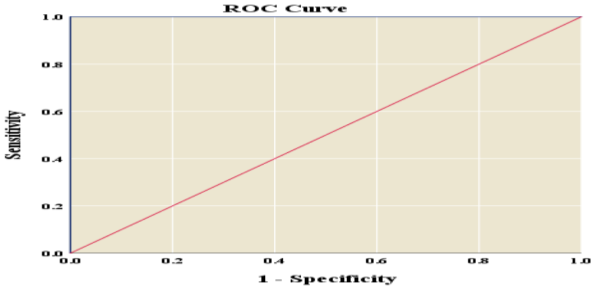

Figure (3.11): ROC curve for the sensitivity and specificity of Zinc / atom (µg/dl) for diagnosis of diabetes mellitus

In this study,the mean differences of Manganese (Atomic absorption) according to study group including (diabetic patients and control group).There were significant differences between means of manganese (Atomic absorption) according to study group as shoun in table (3.8) and figure (3.12).

Table (3.8): The mean differences of manganese (Atomic absorption) according to study group					
Study variables	Study group	N	Mean±SD	t-test	P-value
Manganese / atom (µg/dl)	Diabetic patients	90	0.25±0.03	-79.694	<0.001*
	Control group	90	0.59±0.02		
*P value ≤ 0.05 was significant					

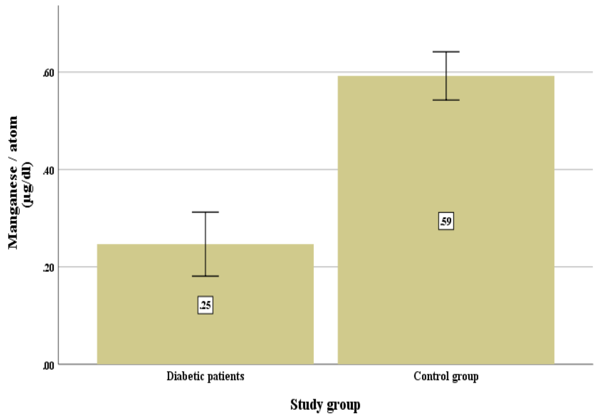

Figure (3.12): The mean differences of Manganese / atom according to study group (P=<0.001)*

In this study , The mean differences of Chromium (Atomic absorption) according to study group including (diabetic patients and control group).There were significant differences between means of chromium (Atomic absorption) according to study group as shown in table (3.9) or figure (3.13).

Table (3.9): The mean differences of chromium (Atomic absorption) according to study group					
Study variables	Study group	N	Mean ± SD	t-test	P-value
Chromium / atom (mg/dl)	Diabetic patients	90	0.20 ± 0.03	-78.184	<0.001*
	Control group	90	0.82 ± 0.06		
*P value ≤ 0.05 was significant					

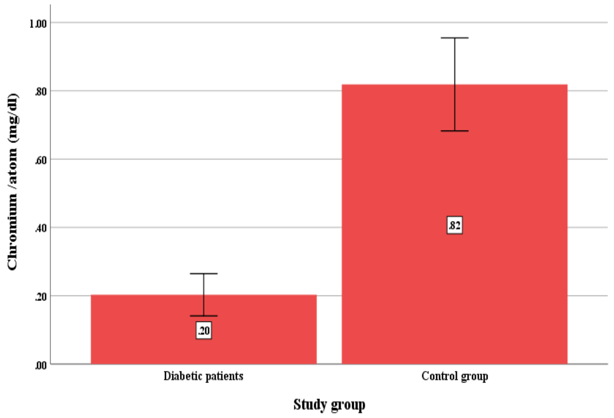

Figure (3.13): The mean differences of Chromium / atom according to study group (P=<0.001)*

In this study, there were no significant differences between means of serum Phosphate (spectrophotometric) according to study group as shown in table (3.10) or figure (3.14).

Table (3.10): The mean differences of serum phosphate (spectrophotometric) according to study group					
Study variables	Study group	N	Mean±SD	t-test	P-value
Phosphorus / sp (mg/dl)	Diabetic patients	90	4.12±0.33	-1.029	0.305
	Control group	90	4.17±0.34		
*P value ≤ 0.05 was significant					

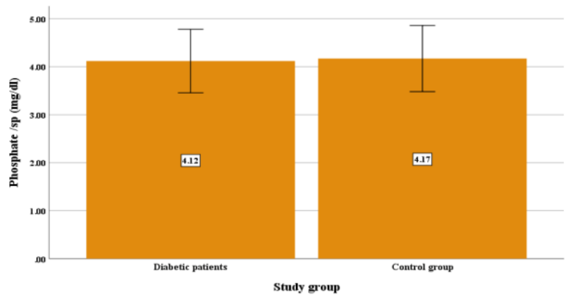

Figure (3.14): The mean differences of phosphate / sp according to study group (P=0.305)

4. Discussion

In the United States, the average age of diagnosis of type 2 diabetes mellitus has reduced from 52 years in the years 1988 to 1994 to 46 years in the years 1999 to 2000.There are a number of probable causes for the ten-year drop.This shift could indicate an earlier start of type 2 diabetes, earlier identification, or a combination of the two. It is uncertain whether the decrease in age at diagnosis was attributable to a reduction in the actual age of onset among American adults or to earlier detection of type 2 diabetes by physicians and this cannot be determined from the data.Decreasing the age at diagnosis could also be the result of increasing public knowledge, which could lead to earlier physician consultations concerning recognized diabetic symptoms, which could represent better population education about diabetes risks [7, 8]. They discovered that being overweight or obese is a substantial contributor to type 2 diabetes and its consequences in both men and women.Both men and women in the overweight range(25 ≤ BMI ≤ 29.99) had a 30% and 10% higher chance of acquiring diabetes, respectively.At(30 ≤ BMI ≤ 39.99), both genders were 100 percent more likely to develop diabetes than those with a normal BMI.These findings point to a larger link between BMI and the onset of diabetes than has previously been shown in similar investigations [9]. Impairment of urea and creatinine level due to increased blood glucose level indicates reduction in kidney function in diabetic patients.Diabetic kidney disease (DKD) develops in about 40% of patients who are diabetic and is the leading cause of chronic kidney disease (CKD) worldwide. Metabolic changes caused by diabetes leads to glomerular hypertrophy, glomerulosclerosis, tubulointerstitial inflammation and fibrosis. Due to this there is large residual risk of DKD.Assessment of serum urea and creatinine impairment with associated factors is very crucial for early diagnosis and prevention of complication of diabetes mellitus [10].In type 2 diabetes individuals, Saharia et al. [11], Basaki et al. [12], Jansen et al. [13], discovered lower zinc plasma concentrations.These findings are linked to a significant quantity of mineral loss in the urine.In these patients, glycemic management influences this loss, which is not balanced by an increase in its uptake by intestinal cells or a reduction in intestinal excretion.Hyperglycemia , according to Jayawardena, interferes with active zinc transport into renal tubular cells, resulting in hyperzincuria [14]. The results of this study approve the previous observations; Zinc concentrations in diabetic type 2 individuals' blood serum are low [15]. AAS is the best technique for the determination of Zn in human blood serum,it is considered by many of benefits such as, simple, rapid, accurate, precise and sensitive, It is highly sensitive, with low limit of detection and less sample volume.Low level of serum Mn in diabetic group in this study .Diabetes was more common in people who had low blood manganese levels, suggesting that manganese may have a role in glucose regulation [16, 17]. When compared to nondiabetic healthy control patients, Rajpathak et al. found that type 2 diabetics had reduced serum chromium levels.In adding to chromium, diabetics' zinc and manganese levels were shown to be lower when compared to the general population [18]. This is consistent with findings from previous research [19]. Another study found that as people got older, their serum chromium levels dropped; this matches the findings of Ding et al. from China, who found that senior diabetics have significantly lower serum chromium levels [20]. Phosphate metabolism disturbances are rather common in diabetic patients.As early in the development of plasma phosphate of diabetic patients levels may be normal or even low; these deregulations may be difficult to distinguish. Although individuals may proceed from initial hyperphosphatemia to hypophosphatemia to normalization of plasma phosphate, phosphate deregulations are more obvious in the context of DKA.A recent study comparing 162 type 2 DM patients to 82 hospitalized non-DM patients found that serum P levels were lower in type 2 DM patients due to metabolic disturbances [21]. In contrast, others found no evidence of lower serum P in the diabetic group compared to the non-DM group in the current study [4]. Another study found no significant differences in calcium and phosphorus levels between diabetics and healthy people [22]. In this study , determine serum phosphate by spectrophotometric method, The simple spectrophotometric method for the phosphate measurement in this work was adapted from the official phosphomolybdate method .This method is much simple, compared with the other developed method.This method provides some advantages such as, simple, easy, cheap and also carried out without any complex preparation sample procedure. Hence, this simple method is suitable for the routine determination of phosphate in common laboratorie [23].

5. Conclusion

1. The current study finding point to a larger link between BMI and the onset of diabetes.
2. Scientific evidences highlighted in present study point out changes in zinc metabolism which contributes to an oxidative stress manifestation in patients with type 2 diabetes mellitus.
3. Low serum manganese is more common among diabetes mellitus type 2.
4. When compared to nondiabetic healthy control patients found that type 2 diabetics had low serum chromium levels.
5. As early in the development of DMT2 plasma phosphate levels may be normal or even low, these deregulations may be difficult to distinguish.
6. Depending on the results obtained, the atomic absorption technique is more sensitive, specific and superior for trace elements determination.

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