The Effect of Type Two Diabetes Mellitus on Superoxide Dismutase (SOD) Activity and its Correlation with HbA1c in Iraqi Patients

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Abstract— Diabetes mellitus (DM) is a group of metabolic disorders, characterized by hyperglycemia resulting from defects in insulin action, insulin secretion or both. Increasing evidence suggests that the oxidative stress plays a role in the pathogenesis of diabetes mellitus and its complications. The current study includes (130) T2DM patients (group P) [51 males and 79 females with an ages range (35 to 55) and ages mean 49.89 years], they are sub-grouped into three categories according to their HbA1c value. Patients with HbA1c less than 7 are considered as good controlled diabetic patients (30 patients) (group P1), while patients with HbA1c between 7 and 8 were considered as medium controlled diabetic patients (40 patients) (group P2), and the patients whom their HbA1c more than 8 are considered as poorly controlled diabetic patients (60 patients) (group P3). The results of patients group were compared with control healthy subjects (35 subjects) (group C) [14 males and 21 Females were with an age range from 35 to 55 years and ages mean 45.51 years]. Patients and controls were characterized in terms of glycated hemoglobin (HbA1c), serum levels of Cu (µg/L), Zn (µg/L), malondialdehyde (MDA) (mmol/L) and superoxide dismutase activity (SOD) activity (U/ml). The HbA1c has been found to be significantly higher in diabetic patients group (P) in comparison to group C. Serum Zn level has been found to be significantly lesser in group P in comparison to group C. Serum Cu level showed an increase in group P, although it is not significance in comparison to group C. Serum SOD activity shown a significant decrease in group P in comparison to group C. Serum MDA level showed a significantly higher value in diabetic patients group P in comparison to group C. The serum Zn was decreased as HbA1c increased i.e. serum Zn level in group C was higher than patients groups, and its value in group P1 higher than group P2 and that was higher than group P3, while serum Cu level was increased as HbA1c increase, i.e. serum Cu level in group P3 higher than group P2 and that higher than group P1, while group C gave the maximum value. The serum SOD activity was decreased as HbA1c increased, i.e. SOD activity was found to be significantly higher in group P1 in comparison with group P2 and P3, while serum MDA level in group P3 was significantly higher than group P2, and P1.

Keywords— EC-SOD, hyperglycemia, MDA, SOD, T2DM.

I.   INTRODUCTION

Diabetes mellitus (DM) is a general term for a group of heterogeneous disturbances of metabolism for which the main result is chronic hyperglycaemia (high blood glucose level); it is usually due to either impaired insulin secretion or impaired insulin action or both [1].

DM can be classified into four categories:

Type 1 DM: It is characterized by β-cell destruction which leads to absolute insulin deficiency; it is usually mediated by immune mechanisms [2].

Type 2 DM: It is usually associated with other health problems such as obesity; it can range from predominant insulin resistance with relative insulin deficiency to prevailing defective secretion with insulin resistance [1& 2].

Gestational DM: It is glucose tolerance impairments that first appear or are first diagnosed during pregnancy [2].

Other specific types of DM: Diseases of the exocrine pancreas (e. g. cystic fibrosis), disease of Endocrinopathies (e. g. Cushing syndrome), drug induced (e. g. glucocorticoids) and genetic defects of the β-cell function (e. g Maturity Onset Diabetes of the Young (MODY)) [1&2].

1.1. Oxidative stress:

Free radicals are atoms or molecules with an unpaired electron and they are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke [4 & 5]. The most common free radicals are superoxide (O_2^-), hydroxyl and ($\cdot OH$)). Free radicals are important intermediates in many natural processes such as cytotoxicity, neurotransmission and phagocytosis [3]. Due to their electron pair, free radicals are very reactive and can react quickly with other molecules to capture the needed electron to gain stability. That is lead to chain reaction, once when it is started its can cascade and finally results a disruption of the living cells [4].

Antioxidants are the molecules capable of stabilizing or deactivating free radicals before they attack cells. Antioxidants are critical for maintaining optimum health and wellbeing. The most common antioxidant systems are enzymatic antioxidant system like superoxide dismutase and glutathione reductase, and nonenzymatic antioxidants system such as C and E vitamins [5].

From the above we can say free radicals are normally formed in the living cells and the body response to the harmful effect of these radicals by its antioxidant systems, so in the ordinary state there is a balance between oxidants and antioxidants, any shift from this equilibrium regardless increasing production of radicals or any defect in the AO systems may be present, that is leads to produce a condition known as ''oxidative stress'' [6]. That plays important role in the development of vascular complications in DM particularly T2DM [7]. Free radical formation in DM by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation leads to damage of AO enzymes [8].

II. MATERIALS AND METHODS

2.1 Study population

Patients enrolled in the present study were subdivided Iraqi T2DM (130) subjects, they clinically diagnosed with T2DM, all of them were diagnosed proven under the supervision of specialists, and (35) healthy subjects as a control. All subjects were selected from the National Diabetes Center for Treatment and Research, Al-Mustassryia University, Baghdad, Iraq during period from November, 2014 to May, 2015.

2.2 Sampling:

After an overnight, fasting venous blood samples were collected aseptically from the subjects via venipuncture, (5ml) was collected and divided into two parts, (4ml) was kept in plain tubes without any anticoagulant at room temperature for 30 min. The tube then was centrifuged ($3000 \times g$) for 10 min. The clear serum was pipetted into clear dry eppendorfs and stored at (-20 C^o) until being used for different investigations, while (1ml) of the whole blood kept in tube with anticoagulant (EDTA) and used in the determination of HbA1c.

2.3 Exclusion criteria:

Any known disease except DM including T1DM and GDM, also patients T2DM and treat with insulin are excluded. The patients who take any supplement are excluded.

2.4 Inclusion criteria:

Any known disease other than T2DM including T1DM and GDM, also T2DM patient and treated with insulin are excluded. The patients who take any supplements are excluded.

2.5 Design:

The patients were sub divided into three groups according to their HbA1c test value. Patients with (HbA1c value less than 7) were considered good controlled T2DM according to universal guide, while, patients with (HbA1c value from 7-8) were involved within middle controlled T2DM. Patients with (HbA1c value more than 8) were included within uncontrolled T2DM.

2.6 Methods:

Glycated hemoglobin (HbA1c) was determined by using kit from Stanbio, Germany. Determination of copper and zinc levels was done using atomic absorption spectrophotometry [GBC 933 Plus, Japan]. Serum MDA was determined using the thiobarbituric acid (TBA) reaction [9]. SOD activity was assayed in serum by quantifying the inhibition of NBT

transformation to formazan, SOD activity was calculated by measuring the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan color development) [10]. The results were expressed as U/ml.

2.7   Statistical analysis

"IBM SPSS (Version 20) was used for data analysis and presentations. Quantitative data were presented as Mean and Standard Deviation (SD). Differences in means between study groups were tested for statistical significance with independent samples t-test. One-way analysis of variance (ANOVA) was used to compare the parameters among groups followed by post hoc test

III.   RESULTS

HbA1c level showed significant (p= 0.000) increase in group P as compared to group C (9.07 vs. 5.40). Serum Zn level showed a significant decrease (p=0.000) in group P as compared to group C (90.28 vs. 72.62), while, serum Cu showed no significant difference in group P as compared to group C (83.00 vs. 83.29). The mean of serum MDA (1.91vs. 1.23), SOD activity (96.15 vs. 146.65) levels of group P showed a significant (P=0.000) increase when compared with that of group C (Table 1).

TABLE 1
MEAN ± S.D IN PATIENTS AND CONTROL GROUPS.

Parameter	Group (C) (n=35)	Group (P) (n=130)	P value
HbA1c (%)	5.4 ± 0.3	9.07 ± 2.4	0.000
Cu (µg/dL)	83.29 ± 16.6	83.00 ± 14.9	N.S.
Zn (µg/dL)	90.28 ± 18.4	72.62 ±17.8	0.000
MDA (mmol/L)	1.232 ± 0.53	1.91 ± 0.55	0.000
SOD activity (U/ml)	146.65 ± 14.59	96.15 ± 19.69	0.000

P<0.05: Significant, N.S. (P≥0.05): Not significant

A significant increase in the mean of HbA1c was observed in groups (P1, P2 and P3) in comparison with that of group C (6.71, 7.66 and 11.21 vs. 5.40%).   No significant increase in mean of Cu serum level in group P1, P2 and P3 in comparison to group C (81.84, 82.15 and 84.13 vs. 83.29), also a mean of group P1 showed no significant decrease as compared to group P2 and P3, and there was a no significant increase in group P3 in comparison to group P2. A significant decrease in the mean of serum Zn level of group P2 and P3 as compared to that of group C (81.69 and 58.16 vs. 90.28), while a no significant decrease in group P1 in comparison to group C (89.43 vs. 90.28). No significant decrease in group P2 as compared P1, a significant increase in group P3 as compared to group P1 and P2 (Table 2). Mean of serum MDA level showed a significant increase in group P1, P2 and P3 as compared to group C (1.88, 1.92 and 1.92 vs. 1.23 mmol/L), also there was an increase (although not significant) in group P3 as compared to group P1. Group P2 showed no significant increase as compared to group P1. Mean of serum SOD activity showed a significant decrease in group P1, P2 and P3 as compared to group C (117.43, 91.73 and 88.46 vs. 146.64 U/ml), and a significant decrease in group P3 as compared to group P1, also a significant decrease was observed in group P2 in comparison to that of group P1, but no significant decrease was observed in group P3 as compared to group P2 (Table 2).

TABLE 2
MEAN (±SD) LEVELS OF HbA1c, SERUM Cu LEVEL, SERUM Zn LEVEL, MDA LEVEL AND SOD ACTIVITY IN C, P1, P2 AND P3 GROUPS.

| Parameter	Group (C) (N=35)	Group (P1) (N=30)	Group (P2) (N=40)	Group (P3) (N=60)	P Value
HbA1c (%)	5.40 ± 0.37	6.71 ± 0.27[***a, ***e]	7.66 ± 0.31[***b,**d,]	11.21 ±2.01[***c, ***f]	0.000
Cu (µg/dL)	83.29 ± 16.64	81.84 ± 15.72	82.15 ± 16.71	84.13 ± 13.56	N.S
Zn (µg/dL)	90.28 ± 18.45	89.43 ± 11.55[***e]	81.69 ± 11.58*b, [***f]	58.16± 11.13[***c]	0.000
SOD activity (U/ml)	146.64 ± 14.60	117.43 ± 18.26***a, ***d	91.73 ± 15.30***b	88.46 ± 15.00***c,***e	0.000
MDA (mmol/L)	1.23 ± 0.53	1.88 ± 0.57***a	1.92 ± 0.43***b	1.92 ± 0.61***c	0.000
SOD activity (U/ml)	146.64 ± 14.60	117.43 ± 18.26***a, ***d	91.73 ± 15.30***b	88.46 ± 15.00***c,***e	0.000

***p<0.001; no asterisk: p≥0.05.

a) Indicate significant difference between groups (C) and (P1).

b) Indicate significant difference between groups (C) and (P2).

c) Indicate significant difference between groups (C) and (P3).

d) Indicate significant difference between groups P1 and (P2).

e) Indicate significant difference between groups (P1) and (p3).

SOD in P1 and P2 groups showed a significant negative correlation with age and MDA. In group P3, SOD showed a significant negative correlation with MDA and HbA1c, while a significant positive correlation was observed with serum Zn(Table 3) and (Figure 1).
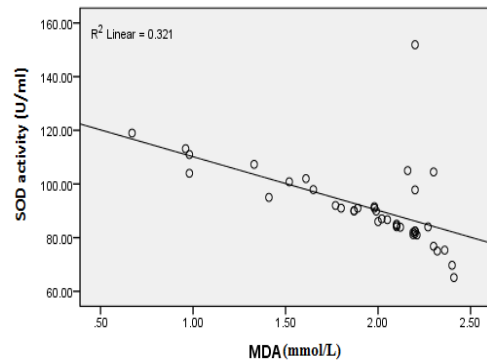
TABLE 3
PEARSON CORRELATION ANALYSIS OF SOD IN GROUP P1, P2 AND P3

Parameters	SOD activity (U/ml)					
	r value group (P1)	P value group(P1)	r value group (P2)	P value group(P2)	r value group(P3)	P value group(P3)
HbA1c (%)	-0.215	0.500	-0.118	0.476	-.397-**	0.002
Cu (µg/dL)	-0.031	0.109	0.241	0.055	0.166	0.205
Zn (µg/dL)	0.071	0.304	0.135	0.283	.302*	0.019
Cr (µg/L)	-0.064	0.455	0.055	0.807	0.399**	0.002
MDA (mmol/L)	-0.662 **	0.001	-0.567**	0.000	-0.827**	0.000

*$p<0.05$; **$p<0.01$; no asterisk: $P \geq 0.05$*
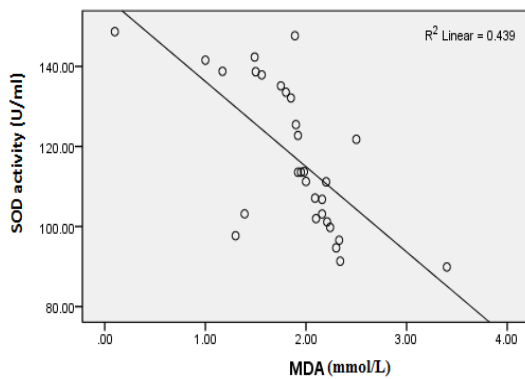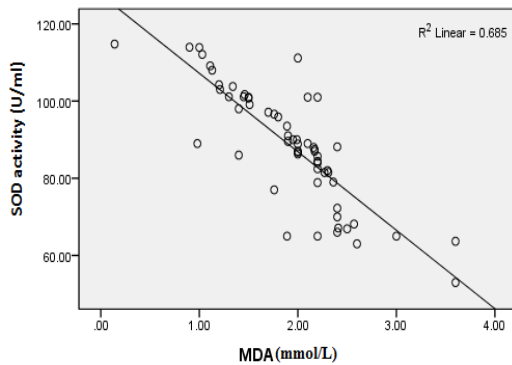

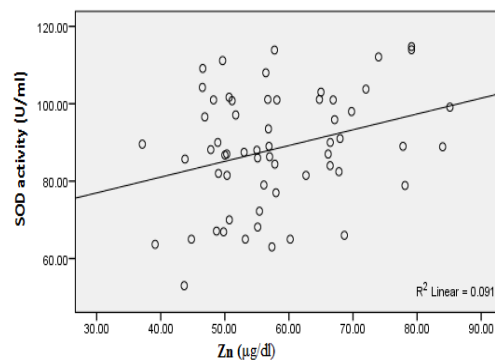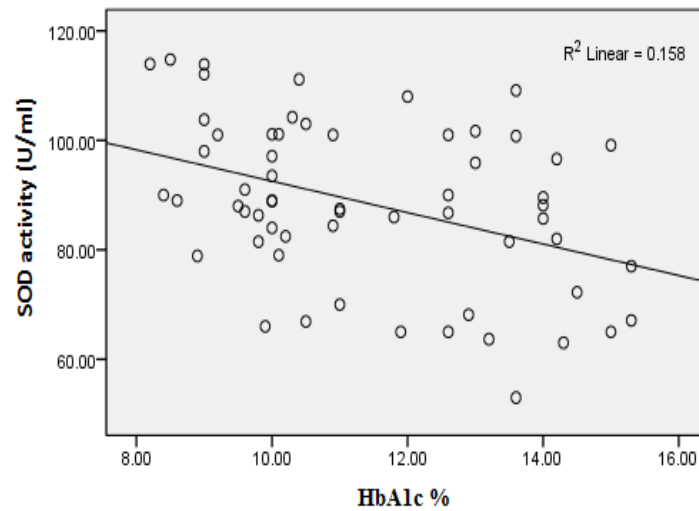**(A) MDA LEVEL IN GROUP P1**

(B) MDA LEVEL IN GROUP P2

(C) MDA LEVEL IN GROUP P3

(D) SERUM ZN LEVEL IN GROUP P3
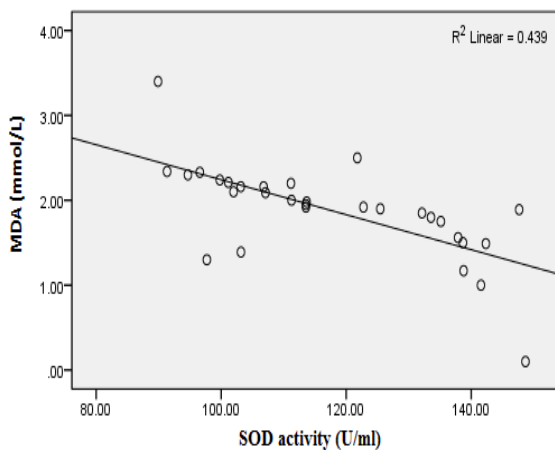
(E) HbA1c IN GROUP P3
FIGURE 1: THE SIGNIFICANT CORRELATION BETWEEN SERUM SOD ACTIVITY

TABLE 4
**PEARSON CORRELATION ANALYSIS OF MDA IN GROUP P1, P2 AND P3.**

Parameters	MDA (mmol/L)					
	r value group (P1)	P value Group (P1)	r value group (P2)	P value Group (P2)	r value group (P3)	P value Group (P3)
HbA1c	.016	0.450	-.119	0.464	.379**	0.003
Cu (µg/dL)	-.294	0.086	-.018	0.914	-.260*	0.045
Zn (µg/dL)	.062	0.054	-.018	0.914	-.446-**	0.000
SOD activity (U/ml)	-.662**	0.000	-.567-**	0.000	-.827-**	0.000

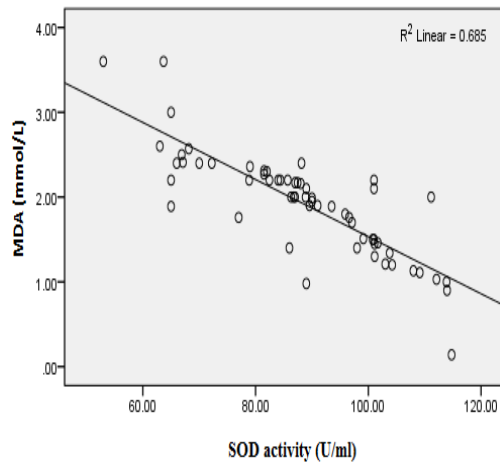*$p<0.05$; **$p<0.01$; no asterisk: $P≥0.05$.*

MDA in group P1 and group P2 showed a significant negative correlation with serum SOD activity. In group P3, MDA showed a significant negative correlation with serum Cu level, serum Zn level, , while a significant positive correlation was observed with HbA1c (Table 4) and (Figure 2).
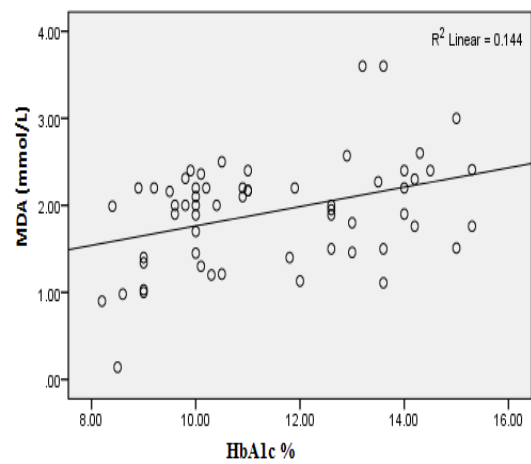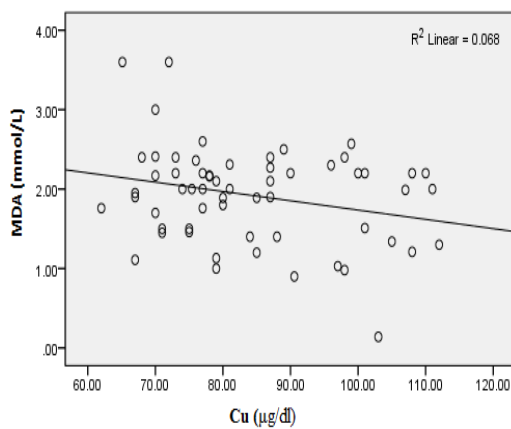

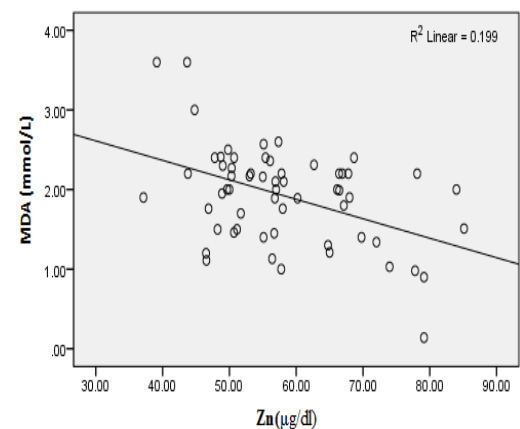**(A) SOD ACTIVITY IN GROUP P1**
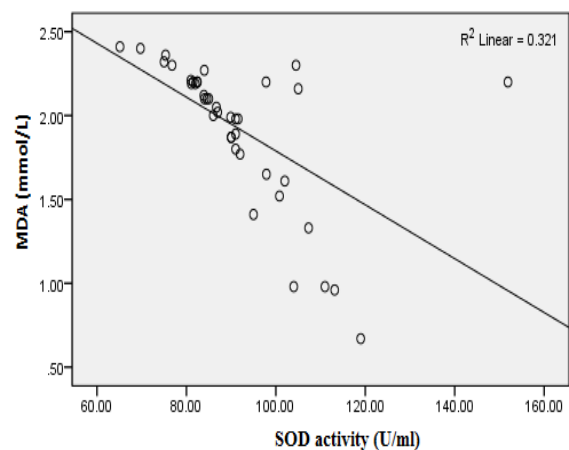
(B) SOD ACTIVITY IN GROUP P2

(C) SOD ACTIVITY IN GROUP P3

(D) HBA1C IN GROUP P3

(E) SERUM CU LEVEL

(F) SERUM ZN LEVEL

FIGURE 2: THE SIGNIFICANT CORRELATION BETWEEN SERUM MDA

IV. DISCUSSION

Trace elements have an important influence on metabolism in the body and any alterations in the trace element levels have been found as either the cause for or the result of disorders like DM and altered insulin metabolism, poor glycemic control and osmotic diuresis may be considered the contributory factors [11].

As shown in Table (2), there was no difference in serum Cu level between diabetic group patients and control subjects, but serum Zn level in patients group were significantly higher than control group C. These findings are in agreement with Mohan Lal, and Sudha K, 2013 [12].

Literatures show that some trace elements such as Zn and Cu play important function in insulin action, including activation of insulin receptor, serving as cofactor or components for enzyme systems involved in glucose metabolism, increasing insulin sensitivity and acting as antionxidant preventing tissue per oxidation [13].

Serum Cu level showed significant increase as HbA1c increase (Table 2). The increase in the Cu levels in patients with DM might be attributed to hyperglycemia that may enhance protein glycation and release of copper ions from copper-containing enzymes. This argument has been supported by M.A. Abou-Seife, 2004, where the level of Cu has been evaluated and its correlation with HbA1c has been determined [14].

An increase in Cu level has been linked to disorders in the structure of the arterial walls, stress, infection and DM. The relation between rising of serum Cu level and the oxidation of LDL-C has been confirmed. But a general consensus exists about the elevated level of Cu, the most important cofactor of oxidative and reductive reactions[15].

A broad wide of experimental and clinical evidence supports alteration of Zn homeostasis in DM. The effects of Zn antioxidant power in diabetes could be due to several mechanisms [16]. Level of Zinc, an activator of insulin, was

investigated in diabetic patients to correlate with diabetic retinopathy. It has been suggested that Zn metallothionine complexes within the islet cells provide protection against immune-mediated free-radical attack and at specific sites where it can compete for iron and copper [17]. Zinc could also help in protecting sulfydryl groups against oxidation and participate in the free radical inhibition in Haber-Weiss cycle by competing with other transition metals. Some investigators found a reducing in serum Zn levels as well as the other investigators like D'Ocon found that serum Zn levels increased in DM [18]. Concerning DM, Zn is considered important mainly because of its major role in the stabilization and the pancreatic storage of insulin [19], Zn is an essential micronutrient which has an important role in the functioning of hundreds of enzymes [20], and work as an efficient antioxidant [21], while oxidative stress is considered to be a main component in initiation and progression of insulin resistance and DM. There are several modes of action have been described to explain the enhanced action of insulin by Zinc. It appears that Zn can have direct insulin-like effects, which may be due to inhibition of the essential glycogen-regulating enzyme GSK3, stimulation of the postreceptor proteins Akt and PI3- kinase, and decrease in cytokines [22 & 23].

Serum Zn level showed a significant decrease as HbA1c increase (Table 2). Zn deficiencies in DM are may be associated with increased free-radical activity and the increased oxidation of lipids, damaging the heart, arteries, and other integral parts of the vascular system [24].

Malondialdehyde is an extremely toxic by-product which is formed in part by oxidation derived from free lipid radicals, and pervious studies have shown considerably raised concentrations in DM. MDA reacts both irreversibly and reversibly with proteins and phospholipids with profound effects [25].

In this study we have observed that (MDA) level as a lipid peroxidation product and a marker of oxidative stress, was highly statistically significant increased between group P and control group (Table 1), this results are in agreement with the findings of Mahreen R. et al, and Amanj Zrar Hassan et. al. [26 & 27]. The most probable causes for the increased MDA levels in serum of diabetic groups may be due to the abnormal lipid metabolism. Hyperinsulinemia and hyperglycemia may enhance the production of free radicals and induce oxidative stress that may also contribute to increased risk for coronary artery disease (CAD) in DM [28]. Hyperglycemia can enhance oxidative stress by several different mechanisms. Autooxidation of glucose and the non enzymatic glycation of proteins generate superoxide $(O2.^-)$ [29]. Wolff SP et al., 1993 stated that the oxidative stress may act as a common pathway to DM itself as well as to its later complications and found significantly higher lipid peroxide levels in diabetic than healthy individuals, matching our study results [30]. Comparative evaluation of the serum levels of MDA was done in patients of diabetes mellitus with and without complications by B.Mandal, et al., 2010. Highly significant increase was found in cases of DM with complications in comparison to cases of diabetes mellitus without complications that indicate more association of oxidative stress with diabetic complications [31]. Free radical interacts in archidonic acid metabolism forming a toxic endoperoxidase. The formed lipid peroxide thus enhances the synthesis of cyclooxygenase, prostaglandin and thromboxane which in turn causes increased platelets aggregation leading to vascular complications [32]. Turk et al., 2002 and Salem, 2010 were observed statistically significant positive correlation in MDA level with HbA1c, our results were also in line with their findings [33 & 34]. MDA also showed statistically significant positive correlation with cholesterol, and negative correlation with HDL-C that agree with M.Salem, 2010 and Nacitarhan et al., 1995 and Nacitarhan et al., 1995 they revealed significantly higher serum MDA level in patients with hyperlipidemic T2DM than in those with normolipidemic DM [35].

MDA showed also negative correlation with zinc level, this finding is in agreement with M. Salem 2010 [35], who was study the correlation of MDA with some trace elements. That is may be due to considering Zn as an antioxidant trace element, thus it could decrease as free radical increase.

Litretures have shown that SOD acts as both an antioxidant and anti-inflammatory in the body, scavengering the free radicals that can lead to wrinkles and precancerous cell changes [36].

The current study showed a highly significant decrease in serum SOD activity of the T2DM patients as compared to healthy subjects (Table 1). These findings are in harmony with earlier studies which involve studding the SOD activity as a marker for oxidative status in T2DM patients [37 & 38].

Beside serum or plasma, SOD activity was wildly studied in erythrocyte in T2DM and the decreasing in erthrocytic SOD activity in the patients group was observed [8 & 39].

The decline in the SOD activity in DM might be due to hyperglycemia which activates various biochemical pathways such as glucose auto oxidation, non enzymatic glycosylation of proteins and activation of protein kinase C, which in turn

overproduce oxidants like superoxide and hydroxyl radicals as well as H_2O_2, or the increase of glycosylated SOD that leads to inactivation of enzyme or loss of its two cofactors Zn^{2+} and Cu^{2+} [40], this is in the harmony with our finding that in diabetic patients, there is a significant correlation between decreased SOD activity and loss of its factor Zn^{2+} and that is in agreement with Hunt JV1991, [41].

Many researches have been studied the SOD activity in DM with and without complications, Bandeira S. M. *et al*., 2012, studied the SOD activity in T2DM patients with and without hypertension and their results were significantly decreased in SOD activity of diabetic subjects and in subjects with diabetes and hypertension compared to the healthy non-diabetic subjects [42].

In diabetic neuropathy, diabetic retinopathy and diabetic cardiovascular disease superoxide dismutase has been studied by J. Kasznicki *et al*.,2012, L.A. Moemen *et al.*, 2014 and Y. Kayama *et al*., 2015 respectively and all of them found that the SOD activity has been decrease in patients subjects as compared to healthy group [43,44 & 45].

V.   CONCLUSION

Type 2 DM patients have a risk of oxidative stress due to increasing the level of serum MDA and decreasing superoxide dismutase activity. And the HbA1c value between 7 to 8 % cannot be considered as an acceptable HbA1c value, due the severing of oxidative stress rising manifested clearly in this patients group.

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