

Estimation of Superoxide Dismutase, Matrix-metalloproteinase-9, and Interleukin -18 in Patients with Type Two Diabetes Mellitus

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Abstract

Antioxidant status imbalance and inflammatory process are cooperative events involved in type 2 diabetes mellitus. This study aimed to investigate superoxide dismutase as a potential biomarkers of antioxidant imbalance, matrix-metalloproteinase-9, and interleukin -18 as biomarkers of inflammation in serum and to estimate the effects of other confounding factors gender, age and finally measuring the relation among the interested biomarkers.

This case - control study included 50 patients, and 45 of healthy subjects matched age - gender were also enrolled in this study as a control group. The focused parameters were measured using ELISA technique. There was significant reduction in the serum superoxide dismutase level and significant elevation in serum matrix- metalloproteinase-9 and interleukin -18 levels which have been associated with diabetes.

This finding may explain the role of the defect in antioxidants status leading to significant reduction in serum superoxide dismutase levels associated with increased inflammatory process leading to significant elevations of a matrix- metalloproteinase-9 and interleukin -18. These parameters added a diagnostic information and evaluated as potential tools for disease risk prediction.

Keywords: Type 2 diabetes mellitus, Superoxide dismutase, Matrix metalloproteinase-9 and Interleukin -18.

قياس سوپر اوكسايڊ دسميوتيز، ماتركس ميتالوبروتينيز-9 وانترلوكين-18 لدى مرضى
السكر من النوع الثاني
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الخلاصة

الخلل القائم في مضادات الأوكسدة والعملية الالتهابية هي أحداث تشارك في النوع الثاني من مرض السكري. تهدف هذه الدراسة الى قياس superoxide dismutase بمثابة المؤشرات الحيوية المحتملة من عدم التوازن المضادة للأوكسدة، وقياس metalloproteinase-9 و interleukin -18 كمؤشرات حيوية للالتهاب، للتحقيق في الآثار المترتبة على غيرها من العوامل الجنس والعمر وأخيراً الكشف عن معامل الارتباط بين هذه المعايير.

هذه الدراسة مصممة لدراسة الارتباط بين المعلومات المحددة في تطور المرض في 50 مريضاً، و 45 معافى سليم مطابقة في الجنس والعمر للتحقق في هذه الدراسة كمجموعة تحكم. تم قياس المعلمات المحدد باستخدام تقنية ELISA. كان هناك انخفاض احصائي ملحوظ في مستوى مصل superoxide dismutase وارتفاع كبير احصائي في مستوى المصل للمعايير metalloproteinase-9 و interleukin -18 التي ترتبط مع تطور المرض.

هذا الاكتشاف قد يفسر دور خلل في وضع مضادات الأوكسدة مما يؤدي إلى انخفاض كبير في مستوى مصل superoxide dismutase في الدم وزيادة عملية الالتهاب التي تؤدي إلى ارتفاعات كبيرة في metalloproteinase-9 و interleukin -18. هذه المعايير أضافت للمعلومات التشخيصية ويمكن ان تقيم كأدوات محتملة للتنبؤ بمخاطر المرض.
الكلمات المفتاحية : مرض السكر من النوع الثاني، سوپر اوكسايڊ دسميوتيز، ماتركس ميتالوبروتينيز-9 وانترلوكين-18 .

Introduction

The pathogenesis of type 2 diabetes mellitus is associated with the induction of the OS that is induced by hyperglycemia⁽¹⁾. High blood glucose level induces OS and decrease antioxidant defenses, thus leading to increase free radical (FR) formation so, there was as a significant difference between patients with T2DM and healthy control group concerning

the total antioxidant capacity between T2DM patients and healthy control⁽²⁾. Several studies have reported the lower concentration of antioxidant enzymes in type 2 diabetes such as superoxide dismutase and glutathione peroxidase^(3,4). Superoxide dismutase (SOD) CuZnSOD EC 1.15.1.1 is one of the most important intracellular antioxidant enzymes⁽⁵⁾.

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Matrix metalloproteinases (MMPs) are a family of zinc-binding proteolytic enzymes that were remodeling the extracellular matrix and then as the response of the inflammatory process pathologically attack the substrates⁽⁶⁾. Increased activity of MMPs has been reported in numerous disease processes. Hyperglycemia and DM enhance expression and activity of MMPs, MMP-9 has been investigated to be increased as a result of the stress hyperglycemia⁽⁷⁾.

Interleukin -18 (IL-18) is a pro-inflammatory cytokine with multiple biologic functions. Elevated levels of IL-18 has been found in the serum of patients with T2DM⁽⁸⁾. From these information, it has been found that immunological and inflammatory processes have an important roles in an initiation and progression of the disease as the same time it was known that inflammatory process plays in etiologic and pathogenic mechanisms for T2DM.

Because of impaired glucose metabolism leads to OS and there is an interrelationship between OS and inflammatory process so, this study was aimed to determine the association of the change in serum levels of SOD, MMP-9, and IL-18 with T2DM development and to assess other confounding risk factors age, gender and finally detect the relation between these studied parameters.

Patients and Methods

This case-control study was designed to examine the associations between the selected parameters with in T2DM patients that was performed at Hawler Medical University, College of Pharmacy on 50 patients of both genders who were randomly selected and newly diagnosed by specialist using clinical examination and confirmed by laboratory tests at diabetic center (Al-Shahida Layla Qassim) and after exclusion of other diseases by clinical history, laboratory investigations and clinical examination. In addition to 45 age – gender matched subjects have been also enrolled in this study as a control group who were also randomly selected. All participants completed the baseline questionnaire concerning several risk factors. This study has been done during the period between October 2013 to December 2014. The control group was confirmed to be healthy by clinical, biochemical and hematological examinations. All clinicopathological data of the patients have been collected from the clinical files.

During the period of this study, the patients and the control groups were not taken anti-inflammatory drugs and antioxidants as

dietary supplements. All procedures were in accordance by the established ethical standards. The protocol of this study has been approved by Ethics Committee of Medical Research at College of Pharmacy / Hawler Medical University. Oral consents have been taken from all the participants before starting the study.

Sample collection

Ten ml of the fasting blood samples have been collected and left for 30 minutes for coagulation purposes and then made centrifugation for 15 minutes at 2500-3500 rpm. The sera of the participants have been separated and divided into several parts and put them into several plastic plain tubes for the biochemical analysis. The sera of the patients and control groups have been stored at (-80 C°) at Medical Research Center /Hawler Medical University till the day of the analysis. The sera have been prepared for measurement by thawing the frozen sera at room temperature. The studied parameters have been estimated using enzyme - linked immunosorbent assay (ELISA) technique at Medical Research Center/ Hawler Medical University.

Statistical analysis

The statistical study has been done using the Statistical Patch for Social Sciences (SPSS vi.18). The results of biochemical tests were expressed as mean \pm standard deviation (SD). Furthermore, student t-test was applied to compare between two means. A (p) value of ≤ 0.05 was considered as statistical significance. Correlations between laboratory findings and continuous variables were evaluated using linear regression analysis.

Results

Subjects characteristics

The clinical characteristics of the patients and control groups have been shown in (Table 1). The patients group has been divided into two groups either as poor control and uncontrolled groups (35 patients with mean age 52.9 ± 7.8 and 15 patients with mean age 63.3 ± 3 respectively) while the control group has been included 45 healthy adults with mean age was 55.9 ± 7.5 .

Effect of controlling the disease on the Serum levels of selected parameters

Serum levels of HbA_{1c}% and glucose were founded to be significantly higher in T2DM patients (uncontrolled group) as compared with poor control and healthy subjects ($p < 0.001$) (Table 1), the serum levels of MMP-9 and IL-18 were increased significantly in uncontrolled group T2DM as compared to poor control and healthy

individuals ($p < 0.001$), while the serum level of SOD was decreased significantly in

uncontrolled T2DM as compared to poor control and healthy subjects ($p < 0.001$).

Table (1): The demographic and clinical characteristics of the studied groups

		N	Mean	±SD	p
Age (Years)	No DM	45	55.933	7.500	< 0.001
	Poor control	35	52.857	7.845	
	Un-control	15	63.267	3.011	
	Total	95	55.958	7.877	
Weight (Kg)	No DM	45	74.222	4.188	.168
	Poor control	35	73.943	7.372	
	Un-control	15	70.400	11.319	
	Total	95	73.516	6.986	
HbA ₁ C %	No DM	45	5.281	.183	< 0.001
	Poor control	35	8.386	.323	
	Un-control	15	10.487	1.499	
	Total	95	7.247	2.097	
FBG mg/dl	No DM	45	92.311	6.338	< 0.001
	Poor control	35	231.486	26.983	
	Un- control	15	297.133	42.963	
	Total	95	175.926	86.010	
MMP-9 ng/ml	No DM	45	1.506	.945	< 0.001
	Poor control	35	1.804	.943	
	Un-control	15	4.563	1.669	
	Total	95	2.098	1.524	
IL-18 pg/ml	No DM	45	48.400	26.375	< 0.001
	Poor control	35	84.240	17.581	
	Un-control	15	116.760	6.603	
	Total	95	72.398	32.953	
SOD ug/ml	No DM	45	501.447	162.781	< 0.001
	Poor control	35	357.320	43.149	
	Un-control	15	171.867	51.409	
	Total	95	396.308	165.428	

$p < 0.05$

Effect of the type 2 diabetes mellitus on the serum levels of selected parameters:

The statistical study has been exhibited that the serum levels of HbA₁C% ,glucose, MMp-9 and IL-18 were significantly increased in patients with T2DM as compared with the

control group $p < 0.001$, while the serum level of SOD was significantly decreased in patients with T2DM as compared with the control group $p < 0.001$ (Table 2).

Table (2): Comparison between the studied groups regarding the focused parameters

	Group	N	Mean	±SD	SE	p
Age years	patients	50	55.980	8.277	1.171	.977
	Control	45	55.933	7.500	1.118	
Weight kg	patients	50	72.880	8.775	1.241	.337
	Control	45	74.222	4.188	.624	
HbA ₁ C %	patients	50	9.016	1.288	.182	< 0.001
	Control	45	5.281	.183	.027	
FBG Mg/dl	patients	50	251.180	44.227	6.255	< 0.001
	Control	45	92.311	6.338	.945	
MMP-9 ng/ml	patients	50	2.632	1.745	.247	< 0.001
	Control	45	1.506	.945	.141	
IL-18 pg/ml	patients	50	93.996	21.297	3.012	< 0.001
	Control	45	48.400	26.375	3.932	
SOD ug	patients	50	301.684	97.041	13.724	< 0.001
	Control	45	501.447	162.781	24.266	

p< 0.05

Gender effect

Concerning MMP-9, there was a significant difference between males and females in patients group p= 0.049 (Table3). Regarding SOD and IL-18, there were no significant differences between men and women in patients group p > 0.05. While,

in both studied groups, concerning MMP-9, there was a significant difference between males and females p=0.049 as well as there were no significant differences between men and women regarding HbA₁C , glucose , SOD and IL-18 p > 0.05 (Table 4).

Table (3): Comparison between males and females concerning the focused parameters in patient group

	Sex	N	Mean	±SD	p
MMP-9 ng/ml	Men	25	2.512	1.840	.049
	Women	25	1.861	1.070	
IL-18 pg/ml	Men	25	70.906	36.060	.665
	Women	25	73.858	29.912	
SOD ug/ml	Men	25	367.226	128.068	.090
	Women	25	424.785	192.370	

p< 0.05

Table (4): Comparison between males and females concerning the focused parameters in both groups

	Sex	N	Mean	SD	P
Age Years	Men	47	55.936	8.641	0.979
	Women	48	55.979	7.141	
Weight Kg	Men	47	74.213	6.504	0.339
	Women	48	72.833	7.433	
HbA ₁ C %	Men	47	7.359	2.348	0.609
	Female	48	7.137	1.837	
FBG mg/dl	Men	47	176.532	90.680	0.946
	Women	48	175.333	82.139	
MMP-9 ng/ml	Male	47	2.409	1.840	0.049
	Women	48	1.794	1.070	
IL-18 pg/ml	Men	47	70.906	36.060	0.665
	Women	48	73.858	29.912	
SOD ug/ml	Men	47	367.226	128.068	0.090
	Women	48	424.785	192.370	

p<0.005

Age effect

There was a significant negative weak correlation between age and SOD in patients group ($r = -0.35$, $p < 0.05$), as much as the age increase the serum level of SOD decrease, while there were a significant positive weak correlations between age and MMP-9 and IL-18 ($r = 0.28$, $p < 0.05$) ($r = 0.26$, $p < 0.05$) respectively (Table 5).

Table (5): The correlation between the age with SOD, MMP-9 and IL-18 in diabetic patients

X variable	Y variable	R	P value	N
Age	SOD	-0.35	<0.05	50
Age	MMP-9	0.28	<0.05	50
Age	IL-18	0.26	<0.05	50

Correlation coefficient**Correlation coefficient between HbA_{1c} and studied parameters**

There was a strong significant inverse correlation between HbA_{1c} and SOD ($p < 0.001$) as much as HbA_{1c} increase SOD decrease which showed more OS ($r = -0.88$, $p < 0.001$). Moreover, there was a strong significant positive correlation between HbA_{1c} and MMP-9 ($r = 0.76$, $p < 0.001$), as much as HbA_{1c} increased the serum level of MMP-9 increased. In addition, there was a strong significant positive correlation between HbA_{1c} and IL-18 ($r = 0.76$, $p < 0.001$), as much as HbA_{1c} increased the serum level of IL-18 increased (Table 6).

Table (6): The relation between HbA_{1c} with SOD, MMP-9, IL-18 in diabetic patients

Y variable	X variable	R	P	N
HbA _{1c}	SOD	-0.88	<0.001	50
HbA _{1c}	MMP-9	0.76	<0.001	50
HbA _{1c}	IL-18	0.76	<0.001	50

Correlation coefficient between studied parameters in patients group.

In patients group, there were a negative strong correlations between SOD with IL-18 and MMP-9 ($r = -0.89$, $p < 0.001$; $r = -0.7$, $p < 0.001$) respectively. In addition, there was a positive strong correlations between MMP-9 with IL-18 ($r = 0.7$, $p < 0.001$) (Table 7).

Table (7): Correlations between the focused parameters in the patients group

X variable	Y variable	R	P value	N
SOD	IL-18	-0.89	<0.001	50
SOD	MMP-9	-0.70	<0.001	50
IL-18	MMP-9	0.70	<0.001	50

Discussion

T2DM and its complications are becoming one of the most important health problems in Erbil population. Therefore, it is necessary to confirm the involvement of novel pathogenic way to provide a good chance for early diagnosis and therapy. The current results has exhibited that antioxidant enzyme SOD and inflammatory markers MMP-9 and IL-18 are useful biomarkers for differentiating diabetic patients from healthy individuals. The results were in harmony with the hypothesis of the current study that, there were a statistical relationship between the significant decreased in the serum level of SOD and significant elevation of the serum levels of MMP-9 and IL-18 with development of T2DM. This study has been detected the existence of inflammation with reduction of antioxidant status in patients with T2DM. The results also suggested that the reduction of the serum antioxidant level may be presented consequence as increased OS associated with T2DM.

Effect of controlling the disease on the serum levels of selected parameters

In uncontrolled patients with T2DM, the serum levels of HbA_{1c}%, glucose, MMP-9 and IL-18 were significantly increased as compared with poor control and healthy individuals (Table 1), while there was a significantly decreased in the serum SOD level in uncontrolled patients with T2DM as compared with poor control and healthy individuals (Table 1). The result of SOD has been exhibited that, OS is severe through uncontrolled stage of the disease which was concordant with the previous findings⁽⁴⁾.

Effect of the Type 2 diabetes mellitus on the serum levels of selected parameters

The statistical study has been revealed that there was a significant decrease in the serum SOD level in patients with T2DM as compared with the control group (Table 2).

It has been published⁽³⁾ that hyperglycemia may interfere with the defensive mechanism antioxidant enzymes which induces the generation of free radicals (FRs). The statistical decrease in the serum SOD level which was significantly related with

the development of T2DM and might be used as a necessary biomarker for diagnosis T2DM.

The current study has been shown an elevated OS as a consequence of overproduction of FRs in patients with T2DM concomitant with the significant decreased in antioxidant enzymes such as SOD which might be associated to the etiology of T2DM and has been used as an important parameters of antioxidant status imbalance and has an significant role in the occurrence of T2DM. The result of the present study was concordant with the previous findings^(3,4).

The explanation for the reduction of antioxidant levels in patients with T2DM because of the that antioxidant enzymes may have been diminished as an attempt to counteract the lipid DNA, protein and lipid damage. Moreover, The increased lipid, protein and DNA oxidation may be as a consequence of a weakened antioxidant defensive system⁽⁹⁾, SOD is considered the first defensive mechanism line against reactive oxygen species (ROS), and it is thought that the activity of SOD might be affected firstly before other antioxidant enzymes as a result of OS⁽¹⁰⁾. Damaged the activity of SOD is due to over production of superoxide -free radicle in addition to the increased of other ROS and induces lipid peroxidation processes in diabetes⁽⁴⁾, moreover, hyperglycemia is associated with loss of Cu^{2+} , which is considered as an important cofactor in SOD activity as well as the activity of SOD is diminished by glycosylation in erythrocytes⁽¹⁰⁾.

The current result indicated that there was a statistical increase in the serum MMP-9 level in patients with T2DM as compared with the control group (Table 2). The result of the present study was consistent with the previous findings^(6, 8-13). The current study also focused the important role of MMP-9 to predict the development of T2DM, in addition, supported the relation between the statistical increase of the serum MMP-9 level with development of the T2DM.

The explanation for this significant elevation in the serum level of MMP-9 in patients with T2DM was that, high blood glucose level directly or indirectly as a consequence of OS and increase glycosylation of products like lipid or protein might elevate the level of MMP-9 and it's activity in large blood vessels⁽¹⁴⁾, moreover, OS may has a necessary role for the pathogenesis of T2DM as well as MMP-9 is activated as a consequence of the FRs generation, which emphasize that antioxidants have a beneficial effects in T2DM therapy.

It is widely accepted that T2DM is related with low grade chronic inflammation⁽¹⁵⁾. So, increased inflammation is considered as non-dependent risk factor for the development T2DM⁽¹⁶⁾.

This study was designed to detect the IL-18 as an inflammatory diagnostic parameter for T2DM. So, this study emphasized that inflammation is associated with increased level of IL-18 by high blood glucose level. There was a statistical increase in the serum IL-18 level as compared with healthy individuals (Table 2) which was in harmony with the previous studies^(8,17,18). According to the current study that, IL-18 might be associated with the pathogenesis of the T2DM as well as the data supported the role of the inflammatory process in the pathophysiology of T2DM. So, this result supported the findings of the previous researches that T2DM might be considered as a low - grade chronic inflammatory status. So, the high serum IL-18 level might expect the development of T2DM and there was a relation between a significant elevation of the serum IL-18 level with the development of T2DM.

Gender -effect

Concerning MMP-9, there was a significant difference between males and females in patients group (Table3). Regarding SOD and IL-18, there were no significant differences between men and women in patients group (Table3). While, in both studied groups, concerning MMP-9, there was a significant difference between males and females as well as there were no significant differences between men and women regarding $\text{HbA}_{1\text{C}}$, glucose, SOD and IL-18 (Table 4). It has been published (4) and illustrated the association between genders and oxidant / antioxidant state, it has been recognized that, the SOD was increased in non-diabetic healthy men compared with non-diabetic healthy women, while in diabetic state, SOD concentration was slightly reduced but there was no statistical significant difference between diabetic men and diabetic women, so the study⁽⁴⁾, has been shown that diabetic patients regardless of the gender, were exposed to an elevated levels of ROS through enhanced lipid peroxidation.

Age - factor

Effect of aging on glucose serum level in type 2 diabetes mellitus

T2DM is a common burden in the elderly⁽¹⁹⁾. Aging is associated with alterations in body composition, which has implications for the development of insulin resistance and diabetes. These information supported the result of the current study that

elderly individuals are at a high risk to develop T2DM. The average age of patients in the current study at diagnosis was 55.98 ± 8.277 years (Table 2). Moreover, the finding of the current study was in harmony with the result of the previous research (20) which has been reported that, the patients with T2DM group with the average age (60-70 years) their mean blood glucose level was 173.4 ± 54.2 mg/dl. Accordingly, there was an association between old age and T2DM.

In addition, other study (21) has been emphasized that an elevated incidence of this disease in elderly subjects might be as result of the variations in the homeostatic pathway that regulates the inflammatory parameters such as C- reactive protein (CRP), IL-6, tumor necrosis factor- α (TNF- α) which are associated with the chronic low grade inflammation occurs in T2DM.

Effect of aging on studied parameters in type 2 diabetes mellitus

There was a significant negative weak correlation between age and SOD in patients group, as much as the age increase the serum level of SOD decrease, while there were a significant positive weak correlations between age and MMP-9 and IL-18 (Table 5). Similarly, (4) reported that, SOD lowers in diabetic age group ≥ 50 years compared with age groups 30–39 and 40–49 years. So, the results of (4) indicated that OS impacts the aging process that may be caused by a number of factors including increased free radical production, decreased antioxidant defense system, or a decreased removal or repair.

Correlation coefficient

The current study reinforced the possibility that high blood glucose level might be associated with elevated level of MMP-9 and HbA_{1c} (Table 6). There was a positive strong statistical relationship between HbA_{1c} and MMP-9 when the serum activity of MMP-9 was increased the amount of HbA_{1c} was also increased. This result was consistent with the finding of the previous research (22). Furthermore, the current result reinforced the possibility that high blood glucose might be associated with reduction in the level of SOD. Table 6 shows, that there was a strong significant negative correlation between HbA_{1c} and SOD, as much as HbA_{1c} increase SOD decrease which showed more OS, this result was shown that antioxidant status are linked with glycemic control, which was consistent with previous result (23). Indeed, there was a significant interaction between elevated glucose and serum IL-18 level.

Data from the current study reinforced the possibility that high blood glucose level might be associated with elevated concentration of IL-18, the data has been shown a positive strong statistical relation between HbA_{1c} and IL-18, when the serum activity of IL-18 was increased the amount of HbA_{1c} was also increased. In addition, in patients group, there were a negative strong correlations between SOD with IL-18 and MMP-9. In addition, there was a positive strong correlations between MMP-9 with IL-18 (Table7). The current study was the first study in Erbil population that investigated the combined effects of antioxidant status by investigating SOD and inflammation by measuring MMP-9 and IL-18 markers in T2DM risk prediction.

Variations in the serum concentrations of these biomarkers associated with occurrence of T2DM and it is important to supply diagnostic information which might help in the management and therapy of T2DM.

Conclusion

The results of the current study detected a statistical decrease in the activity of the SOD in patients with T2DM, accordingly, it was concluded that, T2DM is associated with overproduction of FRs as a consequence of hyperglycemia as well as decreased the activity of the antioxidant enzymes like SOD, which emphasized to supply the therapeutics with antioxidant products. Measuring SOD might be used a parameter to regulate the blood glucose level. In addition, the statistical increase in the concentrations of the MMP-9 and IL-18 as an inflammatory markers emphasized the role of inflammatory process in T2DM risk prediction.

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