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Comparative Study of Human Serum Paraoxonase-1 (PON-1) Activity in Type 2 Diabetes Mellitus: Insights into Oxidative Stress and Antioxidant Defence

Reem M. Azeez[1]*, Wisam Kadhum H. AL-Hashemi[1], Tawfeeq F. R. Al-Auqbi[2]
[1]*Department of Chemistry, College of Science, University of Al-Nahrain, Baghdad, Iraq*
[2]*National Diabetes Center, Mustansiriyah University, Baghdad, Iraq*

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Abstract

Diabetes mellitus is a disease characterized by chronic high blood sugar levels resulting from defects in insulin secretion, insulin action, or both. Prolonged high blood glucose can increase production of reactive oxygen species, leading to elevated oxidative stress. The enzyme paraoxonase-1 (PON1), made in the liver and transported on high-density lipoprotein (HDL), acts as an antioxidant and can counter oxidative stress. This study aimed to compare PON1 activity between healthy controls, obese type 2 diabetics, and non-obese type 2 diabetics. There were 30 healthy controls, 30 obese type 2 diabetics (group I), and 30 non-obese type. Additionally, fasting lipid profiles, fasting plasma glucose, uric acid, albumin, and total bilirubin levels were determined using a clinical chemistry analyzer. The goal was to evaluate differences in PON1 activity as well as metabolic parameters between the three study groups. The results In comparison to the healthy control group, the diabetes group had Significantly higher blood sugar, serum cholesterol, serum triglycerides, serum LDL, and serum uric acid levels, Compared to the healthy control group, PON was Significantly lower in group II patients, Conclusion that Obese type 2 DM patients had significantly lower PON1 and HDL-C levels, which may indicate That the overweight has lessened biochemical functions for these substances. As dyslipidemia, insulin resistance, and high blood pressure are thought to be crucial factors in the cause of metabolic conditions in obese people, the reduced paraoxonase level may increase their likelihood of developing these conditions.

Keywords: Paraoxonase (PON1), 8-hydroxydeoxyguanosine (8-OHdG), Diabetes mellitus, obesity, oxidative stress.

تحليل مقارن لنشاط باروكسوناز المصل البشري 1(PON-1) في اداء السكري من النوع 2: نظرة ثاقبة على الاجهاد التأكسدي والدفاع عن مضادات الاكسدة

ريم محمد عزيز[1]*, وسام كاظم الهاشمي[1], توفيق فاخر العقبي[2]
[1]قسم الكيمياء, العلوم, النهرين, بغداد, العراق
[2]قسم التغذية, المركز القومي للسكري, المستنصرية, بغداد, العراق

*Email: Rauoma.rm93@gmail.com

الخلاصة

داء السكري هو اضطراب أيضي يتميز بارتفاع مستويات السكر في الدم بسبب ضعف إفراز الأنسولين، أو وظيفة الأنسولين، أو كليهما. تم العثور على ارتفاع مستويات السكر في الدم لفترة طويلة، والمعروف باسم ارتفاع السكر في الدم المزمن، لزيادة إنتاج أنواع الأكسجين التفاعلية، مما يؤدي إلى ارتفاع مستويات الإجهاد التأكسدي. إن إنزيم باروكسوناز-1 (PON-1) في المصل البشري، والذي يتم تصنيعه في الكبد ويرتبط بالبروتين الدهني عالي الكثافة (HDL)، يعمل بمثابة إنزيم مضاد للأكسدة له تأثيرات مفيدة في مكافحة الإجهاد التأكسدي. تهدف هذه الدراسة إلى مقارنة نشاط الباروكسوناز 1 (PON1) بين الأصحاء ومرضى السكري من النوع 2 الذين يعانون من السمنة المفرطة ومرضى السكري من النوع 2 غير المصابين بالسمنة. شمل المشاركون 30 شخصًا من الأصحاء، و30 شخصًا يعانون من السمنة المفرطة من النوع 2 (المجموعة الأولى)، و30 شخصًا غير مصابين بالسمنة من النوع 2 (المجموعة الثانية)، ليصبح المجموع 90 شخصًا. تم قياس نشاط PON1 بواسطة ELISA لجميع المشاركين. بالإضافة إلى ذلك، تم تحديد مستويات الدهون في الصيام، والجلوكوز في بلازما الصيام، وحمض البوليك، والألبومين، ومستويات البيليروبين الكلية باستخدام محلل كيميائي سريري. كان الهدف هو تقييم الاختلافات في نشاط PON1 وكذلك المعلمات الأيضية بين مجموعات الدراسة الثلاث. النتائج بالمقارنة مع مجموعة التحكم الصحية، كانت مجموعة مرض السكري لديها ارتفاع ملحوظ في نسبة السكر في الدم، والكوليسترول في الدم، والدهون الثلاثية في الدم، و LDL في الدم، ومستويات حمض اليوريك في الدم، وبالمقارنة مع مجموعة التحكم الصحية، كان PON أقل بشكل ملحوظ في مرضى المجموعة الثانية. استنتاج أن المرضى الذين يعانون من السمنة المفرطة من النوع 2 DM لديهم مستويات PON1 وHDL-C أقل بكثير، مما قد يشير إلى أن زيادة الوزن قد قلل من الوظائف البيوكيميائية لهذه المواد. يُعتقد أن دسليبيدميا ومقاومة الأنسولين وارتفاع ضغط الدم هي عوامل حاسمة في سبب الحالات الأيضية لدى الأشخاص الذين يعانون من السمنة المفرطة، وقد يؤدي انخفاض مستوى الباروكسوناز إلى زيادة احتمالية الإصابة بهذه الحالات.

1. Introduction

Diabetes mellitus (DM) is a metabolic anomaly that is linked with several complications, such as oxidative damage, inflammation, and hyperglycemia. There exist two distinct classifications of diabetes, namely Type 1 and Type 2. Diabetic mellitus of type 2 (T2DM) is the most commonly occurring type of diabetes, characterized by elevated levels of glucose in the bloodstream [1]. The World Health Organization (WHO) identifies diabetes as a major global health concern. In recent years, the incidence of type II diabetes mellitus has increased significantly as a result of metabolic disorders resulting from dysfunctional insulin secretion [2]. Diabetes mellitus type 2 is a condition characteristic of dysfunctional metabolism, which manifests as disruptions in glucose and lipid metabolism. This leads to the collection of modified lipid types in both the tissues and blood circulation, as well as changes in signaling pathways for metabolism that govern pancreatic beta-cell production of insulin [3]. Insulin functions are enhanced glucose performance, lower utilization of glycogen in the liver, reduced glucose absorption in the organ, and increased lipolysis in adipose tissue to raise the levels of circulating free fatty acid (FFA) [4]. There is a well-established link between stress and the development of type 2 diabetes, with stress being recognized as a major risk factor. During the progression of type 2 diabetes, hyperglycemia leads to increased production of free radicals, mainly reactive oxygen species, in all tissues due to protein glycosylation and glucose autoxidation [5]. Elevated oxidative stress in type 2 diabetes has important implications for atherogenesis, including oxidation of lipoproteins, particularly LDL. Moreover, lipid peroxidation of polyunsaturated fatty acids (PUFAs), a free radical-mediated process in vivo, may serve as a useful marker of heightened oxidative stress in diabetes [6]. Obesity raises the risk of contracting diseases like diabetes, inflammatory bowel disease, atherosclerosis, and coronary heart disease. This association is probably accompanied by

oxidative stress abnormalities and possibly metabolic deficits [7]. Increased production of reactive oxygen species in the obese population may cause oxidative damage to cellular proteins and lipids. Oxidative stress is linked to higher levels of obesity and T2DM. Reactive oxygen species (ROS) generation that is high and damaged antioxidant defenses are the hallmarks of these illnesses [8]. many indicators of oxidative stress, including paraoxonase1 (PON1) and 8-hydroxy-2-deoxyguanosine (8-OHdG). It has been thought about using 8-hydroxydeoxyguanosine (8-OHdG) as an oxidative biomarker in Type 2 diabetes has been identified because it shows considerably increased oxidative DNA damage in these patients [9]. The most highly sensitive and practical indicator of DNA oxidative damage is 8-OHdG, which results from the alteration of the DNA base caused by deoxyguanosine's oxidation [10]. The overproduction of ROS can cause lipid, protein, and nucleic acid damage, which can result in apoptosis and cell mutations. As a result, DNA regularly sustains damage and undergoes oxidative modification [11]. In this situation, ROS may result in DNA strand breakage and base alterations. This could involve the most frequently detected DNA damage, 8-hydroxydeoxyguanosine (8-OHdG), which is produced when guanine residues are oxidized [12]. This occurs in mitochondrial and nuclear DNA. Repair is required for DNA damage caused by mitochondrial and nuclear-free radicals as well as cellular damage. However, blood glucose levels and obesity are increasing, which overwhelms the antioxidant system and causes more tissue damage and free radical activity [13].

Paraoxonase (PON) is the name for a family of polymorphic enzymes - PON1, PON2, and PON3 - that exhibit arylesterase activity and are found in human serum. The paraoxonases catalyze the hydrolysis of aromatic carboxylic acid esters as well as organophosphates like the active metabolite paraoxon. Structurally, paraoxonases are glycoproteins containing approximately 337 amino acid residues with a molecular mass around 43 kDa. The genes encoding the paraoxonases are part of a cluster located on chromosome 7 [14]. The enzyme PON is found in a portion of HDL that also contains clustering Apo J and ApoA1, and it is coupled to ApoA1 and attached to a hydrophobic N-terminal portion of HDL [15]. High-density lipoprotein (HDL) particles are connected with it, and it is known to affect HDL's antioxidant and anti-inflammatory functions [16]. PON1 is synthesized in the liver and then distributed to various tissues from the liver. Furthermore, it hydrolyzes the oxidized lipids responsible for inflammation, thus neutralizing their atherogenic effects. found in low-density oxidized lipoproteins [17]. It is a major factor in the HDL molecule's anti-atherogenic action. By acting hydrolytically, this enzyme, which exhibits both PON and arylesterase activity, guards against lipoprotein oxidation and reduces the buildup of lipid peroxides in LDL [18]. The enzyme helps to reduce oxidative damage. PON1 is a critical endogenous free radicals scavenger within the human body [19]. Two Ca^{2+} atoms are also present in the PON1 structure, one of which is located next to a phosphate ion that is essential to the catalytic process near the bottom of the active site gorge. The stability of enzymes is thought to depend on the other Ca^{2+} [20]. Numerous research teams have written outstanding assessments of PON1's extensive studies in human medicine [21]. Initially, the toxicological perspective of this enzyme's protective role against poisoning by organophosphate derivates sparked interest in it. However, more recent studies have concentrated additional clinical aspects, including its role of protection in vascular disease and its use as a disease-related biomarker primarily in three circumstances: (a) oxidative stress because PON1 prevents oxidation [22]; (b) inflammation because PON1 is thought to be a protein negative for the acute phase [23]; and (c) liver conditions, as this organ produces PON1. PON1 can be directly detected by immunological techniques employing particular antibodies and can also be assessed depending on its activity by spectrophotometric assays [24]. As PON1 protects against oxidative stress, its antioxidant properties are believed to correlate positively with insulin

release by the cell in response to high glucose levels. In addition, insulin levels in cells increased in the presence of PON1, suggesting that PON1 plays an important role in insulin biosynthesis. In summary, PON1 seems to promote β cell survival and its normal functional role, which enhances insulin production and secretion [25]. This study aimed to compare paraoxonase-1 (PON1) activity between healthy controls, obese type 2 diabetes patients, and non-obese type 2 diabetes patients.

2. Materials and Methods

2.1. Patient information

This study included 90 participants divided into 3 groups: 30 obese patients with type 2 diabetes, 30 normal weight patients with type 2 diabetes, and 30 healthy control subjects. The inclusion criteria were healthy controls aged 35-65 years and patient groups aged 40-60 years. All participants were between the ages of 35-65 years. The healthy control group had 30 subjects (18 females, 12 males). The obese type 2 diabetes group had 30 patients (19 females, 11 males). The normal weight type 2 diabetes group also had 30 patients (19 females, 11 males). In total there were 56 female and 34 male participants across the 3 study groups. A group of people with obesity and type 2 diabetes was created (BMI ≥ 30), control and T2DM normal weight group (BMI < 27). Before getting blood, all samples were fasted for 12 hours before being collected.

2.2. Sample collection and biochemical analyses

This study was carried out from October 2022 to March 2023 in the Department of Chemistry and Biochemistry, College of Medicine, at the National Diabetes Center, Al-Mustansiriyah University in Baghdad, Iraq. Data was collected from patient medical records, questionnaires administered by an interviewer, physical examinations, and laboratory tests. The questionnaire gathered information on age, gender, diabetes duration, and comorbidities. All participants were screened to exclude any other diseases besides diabetes. The study participants were recruited and evaluated at the National Diabetes Center in Baghdad, Iraq over the 6 month study duration. Each patient's weight and height were recorded to determine their body mass index (BMI). HbA1c readings were taken from the patient's medical history. Laboratory testing was conducted on each patient to obtain FBS, lipid profile, uric acid, albumin, TSB, 8-OHdG, and PON. A sample of 10 ml of venous blood was drawn after 8- 12 hours spent fasting. Blood was centrifuged at 1000 g for 15 minutes after being allowed to settle for 30 minutes before being used, the serum was kept at -20°C. Total cholesterol, HDL, LDL, and TG are all included in lipid profiles. and serum uric acid, total bilirubin, and albumin were measured by a Biosystem analyzer (Spain). PON and 8-OHdG were analyzed using Huma PON ELISA Kit and 8-OHdG ELISA Kit (My BioSource, USA).

3. Statistical Analysis

The results are presented as mean ± standard deviation. Data was analyzed using ANOVA to assess statistically significant differences between the case and control groups, with a p-value less than 0.05 considered significant. The statistical analysis was performed using Prism software. ANOVA was utilized to compare means between the three study groups - obese type 2 diabetes patients, normal weight type 2 diabetes patients, and healthy controls. Statistically significant differences between groups were determined as having a p-value <0.05.

4. Results

Table 1 lists the clinical and biochemical findings for diabetics (obese and normal weight) and healthy control subjects. When compared to controls, obese diabetic participants' mean body weight and BMI were significantly higher. The study found that levels of 8-OHdG,

cholesterol, LDL-C, TG, glucose, and uric acid were significantly higher in obese individuals with type 2 diabetes (group II) compared to non-obese individuals with type 2 diabetes (group I) and healthy controls. Conversely, PON, HDL-C, albumin, and total bilirubin levels were significantly lower in group II compared to the other two groups. Table 2 lists the correlation of PON and 8-OHdG in the two groups T2DM and diabetic obese, the result showed a positive correlation with HDL in the two study groups and a negative correlation between PON and BMI in the diabetic obese group. While the DM group showed a negative correlation with LDL. The other parameter 8-OHdG showed a positive correlation with BMI in the diabetic obese and LDL in the two groups.

Table 1: Comparison of several parameters between T2DM, and obese diabetics with a healthy control group.

Variables	Healthy Controls (n = 30)	T2DM patients (normal weight) (n = 30)	Obese-diabetics patients (n =30)	P-Value
Age (years)	44.20 ± 6.805	47.90 ± 9.953	46.20 ± 7.284	0.4169
Weight (kg)	63.95 ± 9.752	68.43 ± 8.208	102.5 ± 7.310[b,d]	< 0.0001
BMI (kg/m2)	24.36 ± 2.579	24.59 ± 2.061	32.97 ± 2.767[b]	< 0.0001
FBS (mg/dl)	102.7 ± 11.86	150.5 ± 11.52[b]	161.7 ± 11.35[b,d]	< 0.0001
HbA1C (%)	4.960 ± 0.4039	7.627 ± 0.6716[b]	8.053 ± 0.7295[b,c]	< 0.0001
Cholesterol (mg/dl)	145.8 ± 9.835	201.4±10.53[b]	211.8±11.27[b,d]	< 0.0001
TG (mg/dl)	106.5 ± 15.80	160.3 ± 14.70[b]	172.4 ± 14.56[b,d]	< 0.0001
HDL-C (mg/dl	51.47 ± 3.213	42.83 ± 3.842[b]	39.43 ± 3.702[b,c]	< 0.0001
LDL-C (mg/dl)	106.1 ± 4.671	137.9 ± 3.610[b]	142.2 ± 4.164[b,d]	< 0.0001
Uric acid	4.060 ± 0.5934	5.447 ± 0.8003[b]	6.197 ± 0.6584[b]	< 0.0001
Albumin	4.870 ± 0.3816	3.973 ± 0.4025[b]	3.373 ± 0.4076[b,d]	< 0.0001
TSB	0.843 ± 0.0817	0.663 ± 0.0845[b]	0.540 ± 0.0907[b,d]	< 0.0001
PON (nmol/ml)	521.2 ± 32.41	392.6 ± 42.11[b]	353.6 ± 42.43[b,d]	< 0.0001
8-OHdG (nmol/ml)	0.590 ± 0.157	1.634 ± 0.255[b]	2.011 ± 0.268[b,d]	< 0.0001

BMI: Body Mass Index; FBS: fasting blood sugar; LDL-C: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; TG: triglycerides; PON: paraoxonase; TSB: total serum bilirubin, 8-OHdG: 8-hydroxydeoxyguanosine.

[a]p < .05 vs. controls [b]p < .001 vs. controls
[c]p < .05 vs. T2DM [d]p < .001 vs. T2DM

Table 2: Correlation between serum level of PON and 8-OHdG with other variable studies in the T2DM and diabetic obese groups.

Variables	PON		8-OHdG	
	DM	DM-Obese	DM	DM-Obese
	R-value		R-value	
BMI	-	-0.406	-	0.971
LDL	-0.142	-	0.876	0.783
HDL	0.873	0.933	-	-

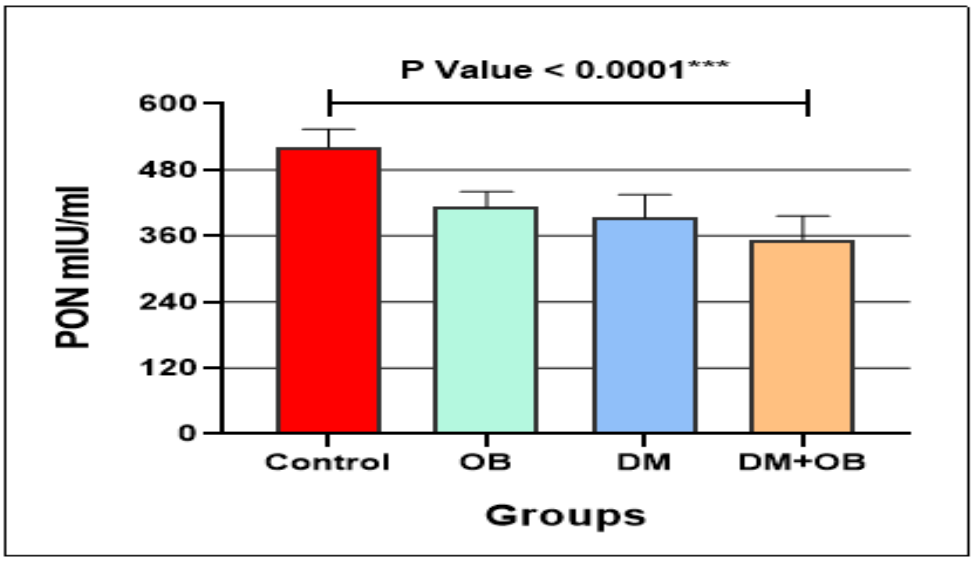

Figure 1: Comparison of PON1 concentration (mIU/ml) between obese, non-obese diabetic, and healthy control.

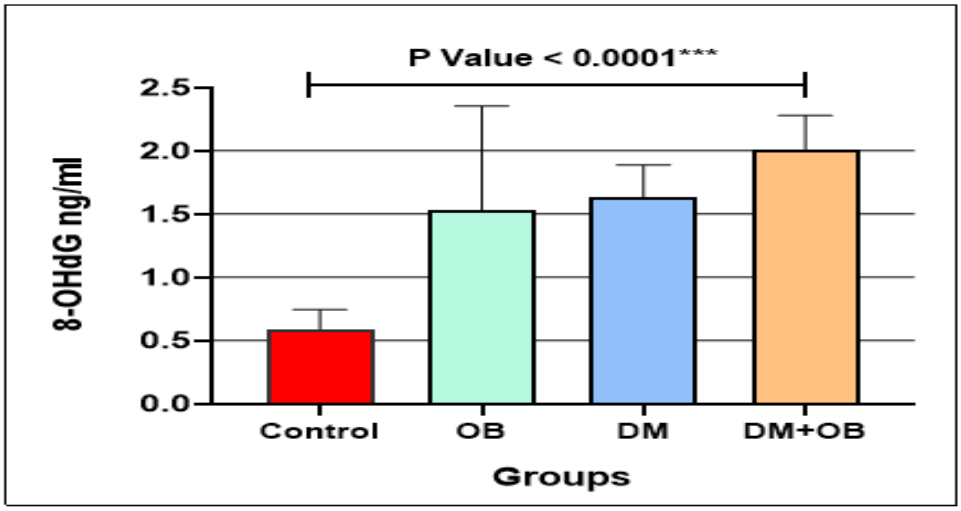

Figure 2: Comparison of 8-OHdG concentration (ng/ml) between obese, non-obese diabetic, and healthy control.

5. Discussion

The current study was conducted to analyze the activity of the PON enzyme among people with diabetes and who suffer from obesity with diabetes compared to healthy subjects, and we found the obese diabetic patient in this investigation had noticeably increased levels of 8-OHdG, FBS, HbA1C, lipid profile, and uric acid. According to a study Type 2 DM patients with problems had considerably lower HDL-C and PON1 activity, which impairs HDL-C function in these patients[26]. A study showed a significantly lower PON-1 activity in type 1 diabetes mellitus (T1DM) [27]. Lowered PON-1 activity was reported in both T1DM and T2DM patients [28]. In comparison to the other study groups, we found that PON1 was considerably lower in obese diabetes participants and who examined oxidative stress in obese and non-obese diabetes patients and found that compared to healthy controls, diabetic patients' ROS levels are higher and their antioxidant levels are lower. Furthermore, compared to diabetic people who were not fat and healthy controls, obese patients with T2DM exhibited increased ROS levels [29]. In diabetic patients, a significant rise in TG and TC and a considerable fall in HDL-C and PON1 activity suggested diabetes dyslipidemia. Particularly,

the patients' drastically reduced HDL-C had an impact on PON1 activity [30]. The findings of lower paraoxonase in the diabetic groups are consistent with several researchers on the serum of diabetes patients that found a decrease in paraoxonase activity utilizing paraoxon as a substrate. Diabetes lowers PON-1 concentrations [31][32]. PON-1 may shield-cells from the cytotoxicity of high concentrations of glucose and may help these cells secrete insulin [33]. The finding that serum paraoxonase 1 level are reduced in diabetes is consistent with prior research showing lower levels in diabetic patients compared to controls. Our results align with these previous studies, demonstrating decreased paraoxonase 1 in both obese and non-obese diabetic groups relative to healthy controls. Furthermore, we found that the obese diabetic group had significantly higher 8-OHdG levels compared to the non-obese diabetic and control groups [34], The oxidative stress marker 8-OHdG reflects DNA damage from oxidative stress as well as overall oxidative stress levels in vivo. Notably, 8-OHdG has been shown to induce apoptotic cell death, which may contribute to tissue damage in diabetes. Therefore, elevated 8-OHdG provides evidence of heightened oxidative stress in obese type 2 diabetic patients [35]. In previous studies, 8-OHdG was found to be one of the commonly used indicators for measuring oxidative DNA damage. These high 8-OHdG levels are quickly brought back to normal following insulin therapy, indicating that hyperglycemia may play a role in oxidative DNA damage [36]. According to the current study, oxidative stress has a significant role in the growth and development of T2DM problems due to higher DNA damage in patients with T2DM when compared to control subjects. The likelihood of DNA oxidative stress damage increases with body mass index [37]. when studying overweight people, discovered that they had much higher 8-OHdG levels than normal-weight people. Our findings in the people who have diabetic imply that other factors, such as physiological and metabolic processes in the body, may result in the creation of ROS, which can result in oxidative damage. These other factors could be involved in oxidative stress in addition to obesity [38]. Both DNA damage in the mitochondria and the nucleus caused by free radicals in the cell must be repaired. But as blood glucose levels rise and obesity rates rise, the antioxidant system becomes overburdened, which causes more tissue damage from free radicals [39].

Our research revealed inversely correlated (negative correlation) relationships between serum paraoxonase 1 (PON1) level with BMI in the obese diabetic group and LDL in the diabetic group (r= -0.406) (r= -0.142) respectively, and positive correlation with HDL (r= 0.933). According to other study, there is a substantial inverse relationship between TC and LDL-C, and PON activity [40]. However, similar outcomes were found by authors investigating, who found a substantial positive connection between PON1 activity and HDL concentration [41]. The additional elements In the diabetic-obese group, serum 8-OHdG significantly positively correlated with BMI and LDL (r= 0.971) (r= 0.783) respectively, but primarily with LDL in the diabetic group (r= 0.876). There are several questions about how BMI and the 8-OHdG are related, having some research demonstrating an opposite link among them [42], while others demonstrate a lack of such an association. In the participants in our study who had diabetes and were obese, we did discover a favorable connection between 8-OHdG and BMI. As a result, the significance of the alterations in the interaction between hyperlipidemia and hyperglycemia in the onset of diabetes in elevated levels of blood sugar. enhanced blood glucose levels and a diabetes diagnosis have an enhanced impact on body weight [43].

Conclusion

Serum PON, 8-OHdG serves as a valuable indicator for assessing the extent of illness or the level of oxidative stress in individuals with obesity and type 2 diabetes who exhibit elevated blood lipid levels. Once glucose levels have reached diabetes status, increased

weight plays a bigger role in the disease's course. This could be used to control cases of high BMI that are linked to blood glucose disturbances. It is noteworthy that the low level of activity of the paraoxinase enzyme is linked to the body's ability to contain fat peroxidation and thus increase the acceleration in oxidative stress. The significant greater decrease in enzyme activity in patients compared to healthy individuals supports the role of this enzyme as an antioxidant. This is because people with diabetes experience increased oxidative stress. Additionally, the association found between disturbances in blood lipid levels and decreased enzyme activity in this study further confirms the connection between the enzyme's antioxidant function and its protective effects on blood lipids from oxidation.

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