RESEARCH ARTICLE

# Assessment of Oxidative Stress Biomarker (8-OHdG) and Paraoxonases 1 in Type II Diabetic Mellitus

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## ABSTRACT

**Objective:** The purpose of this research was to shed light on oxidative damage by determining the concentrations of 8-hydroxy.2 deoxyguanosine (8-OHdG) and the antioxidant enzyme paraoxonase-1 (PON1) at diabetes patients with DM II.

**Methods of Study:** From September 6 to December 4, 2020, (45) patients with diabetes mellitus from (120) people were assessed in the education of Diwaniyah hospital.The study includes measurement of the body mass index,fasting blood glucose level, HbA1c, lipid profile and Levels of oxidative stress parameter 8-OHdG and antioxidant enzyme (PON-1).

**Results:** These findings were made public. The 8-OHdG level in the diabetic group was considerably higher (8.963.96 pg/mL) than in the control group (6.073.31 pg/mL; p 0.01). our observations were appeared In diabetes individuals, there was a substantial increase in blood cholesterol, triglycerides (TG), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) concentration, and a significant drop in high-density lipoprotein (HDL) levels at p.value (0.05) when compared to healthy controls.

**Conclusion:** 8-OHdG level were high at diabetes group than in the healthy groups, suggesting that 8.OHdG could be used as a more sensitive early disease marker. This is important because 8OHdG is a strong indicator of DNA damage caused by oxidative stresses in blood vessels as well as other organs, This increases the risk of heart problems.

**Keywords:** 8.Hydroxy, 2.deoxyguanosines, Oxidatives stresses, Paraoxonase-1, Type II diabetic.

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**Conflict of interest:** None

## INTRODUCTION

Type II diabetes (T2Ds) is a metabolic illness marked by insulin resistance and insufficiency, leading to chronic hyperglycemia and the risk of kidney, neurological, and cardiovascular problems.[1] Overweight and advanced age are risk factors for the development diabetes I T2D-related morbidity and mortality are mostly caused by long-term vascular consequences.

Dyslipidemia oxidative stress, and activated leukocytes are all metabolic dysfunctions related with kind II diabetes. development of T2D problems has been linked to oxidative stress and leukocyte activation.[2] The breakdown of vital components caused by free radicals, oxidative stress can cause cell damage and dysfunction.[3]The activation of nuclear transcriptional active factor (NF)-kB by oxidative stress has been demonstrated, subsequent in deoxyribonucleic acid (DNA) destruction. Studies have revealed an increase in oxidative Damages of DNA.[4] Regarding 8-OHdG, buildup in patients with type II diabetes, implying that hyperglycemia plays a role in oxidative damage of DNA. The result of oxidative damage of DNA is 8-OHdG that results from particular enzymatic cleavages following 8-hydroxylition of the ganines bases. It's a good indicator of oxidative damage of DNA, and it's easy to test with enzyme-linked immunoassay (ELISA).[5]

An imbalance exists between the generation of free radicals and the generation of conservatives, which is frequently enhanced by malfunctioning mitochondria, and antioxidant defenses leads to oxidatives stress at secretion cells of insulin, Endothelial cells and insulin-sensitive peripheral cells, the mitochondrial active transport is a key generator for reactive species of oxygen (ROS).[6] Diabetic complications, oxidative stress is created, and it is believed to have a part in the evolution of diabetes related pancreatic beta-cell dysfunction.[7]

The etiology of type II diabetes and its consequences has long been linked to oxidatives stress.[8] In T2D patients,

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The antioxidant defense system is weakened by metabolic imbalances, which lead to oxidative stress. There appears to be an imbalance between the oxidant and antioxidant systems in T2D patients.[9]

Human blood paraoxonase-1 (PON1) is a liver-produced enzyme that binds to HDL in the bloodstream. It is an antioxidants enzyme that catalyzes the hydrolysis of damaging hydrogen peroxide in oxidized lipids in both LDL and HDL, preventing atherosclerosis and cardiovascular disease.[10] When compared to people with high PON-1 activity, those with low PON-1 activity are more susceptible to disorders involving increased oxidative damage and lipid peroxidation. Circulating blood paraoxonase (PON1), a 43-kDa protein, catalyzes the hydrolysis of organophosphate esters, aromatic carboxylic acid esters, and carbamates.[11] PON1 is mostly related It is made from high lipoprotein and is produced in the liver HDL.[12] Due to its ability to remove hydroperoxides, the enzyme reduces lipid peroxide production in LDL and attenuates the biological consequences of slightly oxidized LDL.[13] In cardiovascular disease and diabetes mellitus, PON1 activity was observed to be reduced.[14] These alterations could be influenced by a number of reasons to begin with, oxidative stress is exacerbated, and lipid peroxidation may play a role in vascular wall deterioration.[15] Second, in diabetes, glycation of proteins, particularly enzymes, may reduce their activity.[16] The esterolytic PON1 activity was preserved when HDL were incubated at very high glucose concentrations 1 mol/l. The esterolytic PON1 activity of HDL protected in normal (5 mmol/l) or increased (up to 100 mmol/i) glucose levels was lost.[17]

Reduced PON1 activity is thought to be a significant source of defective HDL in type II diabetics, that has been linked to faster atherosclerosis and, as a result, higher CAD mortality.[18] Given the significance of hyperglycemia in directly generating oxidative stress through the depletion of endogenous anti-oxidants and the facilitation of reactive oxygen species formation, it has been hypothesized shows the beneficial effects of PON1 activity on LDL particle peroxidation are much more significant in type II diabetes patients than those in non-diabetic people.[19]

As a result, the study's goal was to establish the amount of oxidative DNA damage in patients with type 2 diabetes by measuring 8-OHdG levels and determining PON-1 activity and its interaction with lipid profile in order to provide further insight into therapy indications.

## METHODOLOGY

The present study was conducted on 90 females and males patients age ranged 30 to 60 years were examined from 6th September to the 4th December 2020 in the education Al-Diwaniyah hospital in the Diwaniyah provenance, five milliliter of blood from each patients were taken from radial vein. Biochemical testes were including measurement of fasting bood glucose level and HbA1c. Also lipid profile were measured as well as serum 8-OHdG and PON-1were assessed, the samples divided into: Fourty five patients with T2D and 45 controls people who were considered the control group and 30 patients were excluded from the presence of other diseases. The study involved 21 male and 24 female patients, 25 male and 20 female controls.Patients were diagnosed by specialist physicians based on some clear clinical parameters and confirmed by some laboratory analysis

## RESULTS

Table 1 illustrate the patient and control group distributions (1). entire of 45 diabetes participants in this study were patients., with 21 (46.7%) males and 24 (53.3%) females, as well as 45 healthy controls.

- The There were no important differences in the mean age of the participants. among diabetic patients 54.4411.190 years and the control group 49.2114.91 years ($p = 0.057$)
- The patients (men and women) were separated into three groups based on their diabetes duration: or less 5 years (48.9%), 5 to 10 years (28.9%), and more than 10 years (10.1%). (22.2 percent).
- The normal weight 6 (13.3 percent) body mass index (BMI) group had the highest percentage of BMI groupings. overweight 18 (40.0%) and obesity 21 (46.7%).
- BMI (body mass index) In diabetes patientsIn terms of BMI, there were considerable disparities (BMI) of 30.505.71 $Kg/m^2$ ($p < 0.001$) when compared to 26.675.28 $Kg/m^2$ in the control group.

### Diabetic Mellitus Patients and Control Groups were Compared

Table 2 shows the outcomes, which include:

- Blood glucose level after fasting:

The results showed that diabetic patients had a significantly higher fasting blood glucose (FBG) level of 233.80108.65 mg/dL ($p < 0.0001$) in conmpare with the control group of 91.2914.65 mg/dL

### The A1c Level of Glycosylated Hemoglobin

- HbA1c levels were found to be significantly higher in diabetes individuals (9.392.13%, $p = 0.001$) when compared to the control group (5.270.68%).

### Level of Serum Lipid Panel

The results of serum lipid profile levels showed significant increase in serum total cholesterol level ($p = 0.02$),TG ($p = 0.0001$), LDL-C ($p = 0.01$) and vLDL ($p = 0.02$) in diabetic patients in comparing with control group,but the levels of HDL-C are significantly decreased ($p = 0.001$) in diabetic patients when comparing with control group.

### Biomarker Level Between Patients and Healthy Controls:

The results that shown in Table 3 including :

- 8-OHdG
- At type II diabetic patient, 8-OHdG levels are higher than in control subjects (Figure 1).
- PON-

The results showed that PON1activity is significantly lower in patients with T2D $0.75 \pm 0.69$ at ($p=0.0001$) compared to control group $1.79 \pm 1.62$ (Figure 2).

**Comparison of Biomarkers levels between Male and Female patients**

The Comparison of Biomarkers levels between Male and Female patients in (Table 4**)** indicated**:**

That the mean of 8-OHdG was higher in males when compared with females but not significant (p=0.9) (Figure 3), while the mean of PON1 was higher in male when compared with females with significantly (p = 0.02) (Figure 4)

**Table 1:** Demographic of study groups patient and control

| | *Mean ± SD* | | |
|---|---|---|---|
| *Variables* | *Patients N.=45* | *Controls that are healthy N.=45* | *p-values* |
| Age (years) | 54.44 ± 11.190 | 49.21 ± 14.91 | 0.057 |
| 28–38 year n (%) | 4 (8.9%) | 18 (40.0%) | 0.061 |
| 39-49 year n (%) | 11 (24.4%) | 10 (22.2%) | |
| 50-60 year n (%) | 14 (31.1%) | 9 (31.1%) | |
| > 60 year n (%) | 16 (35.6%) | 8 (35.6%) | |
| Male n (%) | 21 (46.7%) | 25 (55.6%) | 0.655 |
| Female n (%) | 24 (53.3%) | 20 (44.4%) | |
| BMI | 30.50 ± 5.71 | 26.67 ± 5.28 | 0.001 * |
| Normal weight n (%) | 6 (13.3%) | 16 (13.3%) | 0.015 for patients |
| Overweight n (%) | 18 (40.0%) | 18 (40.0%) | |
| Obesity n (%) | 21 (46.7%) | 11 (24.4%) | |
| Disease duration groups | 6.38 ± 5.18 | 0 | NA |
| < 5 year n (%) | 22 (48.9%) | 0 | 0.074 for patients |
| 5-10 year n (%) | 13 (28.9%) | 0 | |
| > 10 year n (%) | 10 (22.2%) | 0 | |

significant differences at p < 0.05. NA: non applicated

**Table 2:** Patient and health-control biochemical markers are compared

| | *Mean ± SD* | | |
|---|---|---|---|
| *Variables* | *Patients N=45* | *Healthy controls N=45* | *p-value* |
| FBS (mg/dL) | 233.80 ± 108.65 | 91.29 ± 14.65 | 0.0001 * |
| HBA1C% | 9.39 ± 2.13 | 5.27 ± 0.68 | 0.0001 * |
| Cho (mg/dL) | 167.14 ± 49.83 | 193.64 ± 62.42 | 0.029 * |
| T.G (mg/dL) | 210.31 ± 109.50 | 134.16 ± 55.11 | 0.0001 * |
| High density lipoprotine (HDL) (mg/dL) | 39.48 ± 9.38 | 46.60 ± 9.29 | 0.0001 * |
| Low density lipoprotine (LDL) (mg/dL) | 85.49 ± 42.62 | 115.88 ± 66.38 | 0.012 * |
| VLDL (mg/dL) | 42.01 ± 21.96 | 30.35 ± 24.75 | 0.020 * |

**Table 3:** Biomarker level between patients and controls

| | *Patients N.=45* | | *Healthy controls N.=45* | | |
|---|---|---|---|---|---|
| *Variables* | *Mean + SD* | *Median + IQR* | *Mean + SD* | *Median + IQR* | *p-values* |
| 8-OHdG (ng/mL) | 8.96 ± 3.96 | 8.21 (6.64–9.81) | 6.07 ± 3.31 | 5.55 (3.40–8.32) | 0.0001*# |
| PON-1 (ng/mL) | 0.75 ± 0.69 | 0.55 (90.24–0.99) | 1.79 ± 1.62 | 1.09 (0.63–2.84) | 0.0001*# |

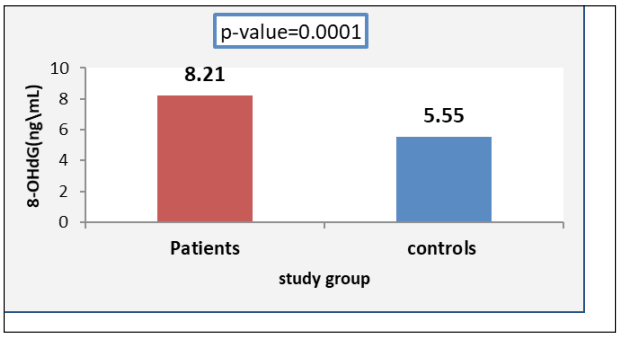


**Figure 1:** 8-OHdG level between patients and controls

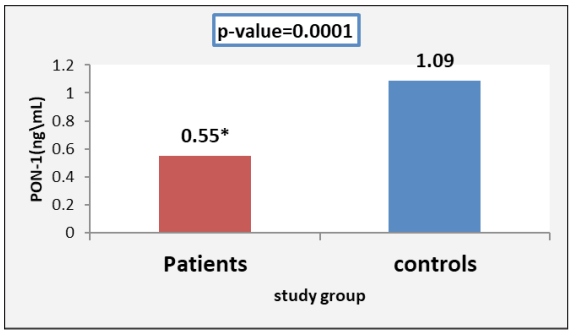


**Figure 2:**PON1 level between patients and controls

**Table 4:** Comparison of Biomarkers levels between Male and Female patients

| | *Male N=21* | | *Female N=24* | | |
|---|---|---|---|---|---|
| *Variables* | *Mean + SD* | *Median + IQR* | *Mean + SD* | *Median + IQR* | *p-values* |
| 8-OHdG (ng/mL) | 9.06 ± 3.83 | 6.95(8.02–10.05) | 8.87 ± 4.16 | 6.47(8.24–9.84) | 0.909 |
| PON-1 (ng/mL) | 0.99 ± 0.84 | 0.40(0.92–1.44) | 0.53 ± 0.43 | 0.21(0.46–0.85) | 0.022 |

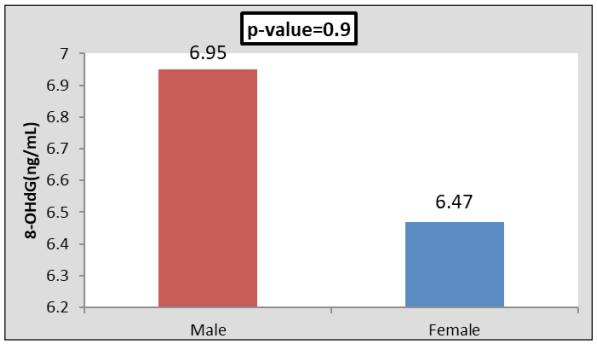


**Figure 3:** 8-OHdG levels between Male and Female patients

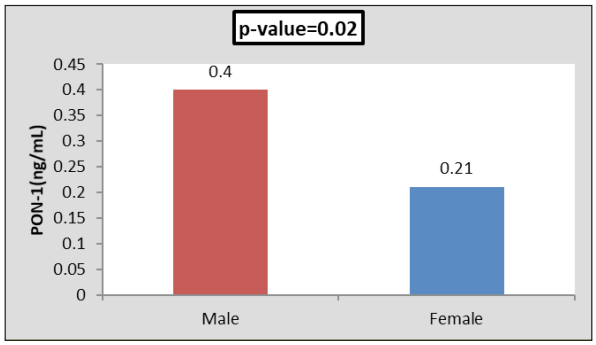


**Figure 4:** PON-1 levels between Male and Female patients

## DISCUSSION

According to our research, diabetic patient has extra severes oxidatives tension in conmpare to health people. Increased free radical production or a defective anti-oxidant defense structure are two possible reasons of increased oxidative stress. Diabetes was discovered to have higher quantities of free radicals.[20] Due of their great responsiveness and propensity to respond, to create covalents connections non--enzymatically, oxygen-free radical are harmful to tissue. Extensive research with biological materials has demonstrated that reactive free radicals can cause chemical changes in cells and harm proteins, lipids, carbohydrates, and nucleotides. Antioxidant defense systems in cells scavenge radicals of oxygen and inhibit radical chains and lipid per-oxidations to counteract these effects.[21]

In type II diabetes patients and normoglycemic participants, we looked at oxidatives stresses markers. Fasting blood sugar, A1C, and an oxidatives stresses biomarker (8-OHdG) were all significantly affected in diabetic patients. According to the findings, both male and female patients had considerably higher fasting serum glucose levels than healthy subjects, and their A1C percentage was higher than recommended. Such results was reached despite the fact that patients had type II diabetes were being treated with hypo-glycemic medication, and they were most likely related to inadequate diabetic control.

PON action in diabetes patients on average was considerably lower than in controls participants in this study. PON1 action may be decreased at type II diabetic for a variety of reasons. This could be due to the enzyme's structural alterations as a result of gluco-xidant or lipo-xidant Feretti etal. discovered which incubating HDL with glucose condensed, In vitro action of HDL-associated paraoxanases, implying that glycosylad HDL causes physiochemical changes in HDL characteristics. Hedrick *et al.* also discovered that in-vitro glycosylation of pure paraoxanase proteins reduced its enzymatic activity by 40% and The capacity of HDL to discharge and stabilize PON1 from cells may be impaired by diabetes-induced reductions in HDL molecular weight and cholesterol buildup in HDL particles.[22] PON-1 activity decreases prohibit HDL from performing its antioxidant function, speeding up atherogenesis and its consequences.

Increased insulin resistances and the progression of beta-cell malfunction are thought to be the causes of type II diabetes.[23] Failing of insulin-stimulated glucose absorption by fat and muscle leads glucose levels in the blood to continue to increase in people with diabetes or insulin resistances.The degree of beta-cell breakdown determines the rise in blood

sugar levels As a result, glucose absorption by insulin-resistant tissues rises. Multiple interacting nonenzymatic, enzymatic, and mitochondrial mechanisms boost oxidative generation while impairing antioxidant defenses when glucose flux is increased.[24] Finally, we believe that diabetes patients' oxidative stress is influenced by glycemic management.

## CONCLUSIONS

This present study was revealed, oxidative DNA damage was high in patient with type II diabetic. Type 2 diabetics are much more prone to suffer from oxidative stress., which can lead to problems. According to our research, that decreased PON-1 production in T2D is a female-only phenomena, Women are more afflicted than men. This disparity may explain, at least in part, how diabetic women are far more likely than diabetic men to have cardiovascular problems. The decrease in PON1, reflecting in a poorer HDL functional quality, could represent a target for nutraceutical or pharmacological treatment in both sexes, but in particular in women The substantial association between HbA1c and oxidative stress measures suggests that adequate glycemia control can reduce oxidative stress and hence ease long-term diabetic problems.

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