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Evaluation of Paraoxonase-1 Activity of Arylesterase and Lactonase and Their Correlation with Oxidative Stress in Children with Type 1 Diabetes Mellitus

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Abstract

Background: Type 1 diabetes mellitus (T1DM) is a chronic autoimmune condition that can lead to long-term complications due to oxidative stress and metabolic dysregulation. Paraoxonase-1 (PON-1), an enzyme associated with high-density lipoprotein (HDL), has dual activities: arylesterase and lactonase. These activities protect lipids from oxidative damage. The functional status of PON-1 in children with T1DM may provide insights into the relationship between oxidative stress and the enzyme's protective role. This study aims to assess the arylesterase and lactonase activities of PON-1 in Iraqi children with T1DM.

Methods: Sixty-seven children with T1DM were enrolled and compared with 57 age-matched healthy controls. The enzymatic activities of arylesterase and lactonase were measured to evaluate PON-1's functional status. The Paraoxonase-1/HDL (PON/HDL) ratio was calculated to assess lipid protection and antioxidant capacity. Oxidative status was assessed by measuring total oxidative status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI).

Results: PON-1 activity analysis showed a significant reduction in arylesterase (2.36 ± 1.17) and lactonase (21.9 ± 7.31) in the patients group compared to controls (arylesterase=4.54 ± 1.84, lactonase =29.51 ± 9.92). TOS and OSI were significantly higher, while TAS was significantly lower in the patients group. Pearson correlation revealed a positive correlation between HDL-C and arylesterase ($P = 0.002$, $r = 0.379$), and HDL-C and lactonase ($P = 0.040$, $r = 0.366$).

Conclusion: Reduced PON-1 activity is associated with T1DM, suggesting that enhancing PON-1 or reducing oxidative stress may help prevent diabetic complications and improve cardiovascular health.

Keywords: Antioxidant Activity, Paraoxonase-1, Arylesterase, lactonase, Oxidative Damage, Type I Diabetes Mellitus.

Introduction

Type 1 Diabetes mellitus (T1DM) is a chronic autoimmune disease that occurs when the immune system attacks the beta cells in the pancreas as foreign bodies. Consequently, the body loses its ability to use sugar as an energy source, which leads to its accumulation in the blood. This disease is affected by many environmental as well as genetic factors. This complex disease requires precise and sustainable management. Therefore, ongoing scientific research is essential to discover new diagnostic and therapeutic methods to combat this disease (1-4).

Paraoxonase1 (PON1) (EC: 3.1.1.2, 3.1.1.81, and 3.1.8.1) is a calcium-dependent glycoprotein consisting of 355 amino acid residues and has a molecular weight of 43 kDa (5,6). Upon synthesis in the liver, PON1 is secreted into the bloodstream, primarily associating with high density lipoproteins

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(HDL), with minor associations with very low-density lipoproteins (LDL) and chylomicrons (7). PON1, myeloperoxidase, and HDLs create a functional alliance through mutual binding. Notably, unbound PON1 exhibits reduced enzymatic activity compared to its HDL-bound counterpart (8). PON1 is shuttled from the liver to diverse tissues where it attaches to cell membranes, providing protection against lipid peroxidation (9). Additionally, PON1 serves to counteract LDL oxidation and mitigate the inflammatory response (10), display only a fraction of the PON1 levels found in adults at birth, equivalent to one-third of adult levels. It takes approximately two years for them to attain the same PON1 concentrations observed in adults. This underscores children's heightened susceptibility to organophosphate exposure (11). The aim of this study is to assess the levels of two PON1 activities (arylesterase, and lactonase) as indicators of antioxidant levels in children with T1DM and to evaluate the overall oxidative stress status.

Materials and Methods

Subjects

A case –control study was conducted after obtaining the approval from the College Council of Science, Department of Chemistry, University of Baghdad as well as from the Research Ethics Committee at the College of Science Approval for scientific research was granted by the Research Ethical Committee approved on February 6, 2024, Ref.: CSEC/0224/0017, which has been endorsed by the Iraqi ministries of Environment, Health, Higher Education, and Scientific Research. The parents' approval was obtained before collecting blood samples from children in the Medical City Child Protection Hospital, which is located in Baghdad/ Iraq. The study group comprised 124 subjects including 67 (male=28, female=39) children and adolescents with insulin-dependent diabetes with age range (1-17 year). A control group of 57 subjects (male= 23, female = 34), aged 1–19 years, was enrolled. According to doctor's prescription; the patients were given two types of pure insulin which was used before meals to help the body break down sugar present in the next meal, and the other is insulin to which auxiliary substances have been added and given to the patients who needed stable blood sugar levels throughout the day. The mean± SD of age in patients group and control group was (9.71±3.62) and (10.78 ± 3.80) respectively.

Exclusion Criteria

Recent Food Consumption: Participants who had consumed food within 8 hours prior to sample collection were excluded. This is in line with the American Diabetes Association (ADA) guidelines 2023, which recommend fasting for a minimum of 8 hours prior to blood sampling to ensure accurate metabolic assessments (12).

Elevated Bile Levels: Individuals with elevated bile levels in the blood (cholestasis) were excluded due to their potential impact on liver function and lipid metabolism, which can affect the study results (13). Kidney Disease: Patients with any form of chronic kidney disease (CKD) were excluded. This exclusion criterion aligns with the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines 2020, which caution against including patients with CKD due to the confounding effects of impaired renal function on oxidative stress and enzyme activity (14).

The blood samples were obtained via venipuncture. Samples were centrifuged after clotting at 590 x g for 10 minutes. Serum was separated and stored in Eppendroff tubes at -70 °C until used for analysis.

Measurements of some clinical parameters

The determination of fasting blood glucose (FBG), Cholesterol total (TC), Triglycerides (TG), High-Density Lipoprotein- cholesterol (HDL-C), urea, creatinine and total protein levels were determined by employing the enzymatic colorimetric methods using kits from Bio-systems Company: Medtronic (USA) operating in an end-point mode which was monitored by spectrophotometer (SP-300; manufactured by OPTIMA Inc., Japan).

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As for the concentration of Low-Density Lipoprotein Cholesterol (LDL-C) and Very Low-Density Lipoprotein Cholesterol (VLDL-C), Friedewald's formula (15) was used to calculate their concentration.

Arylesterase activity of PON1

The enzymatic analysis was made according to Shen *et al*.,(2014) method using phenyl acetate as substrate (16) which can be summarized in the following: the assay solution contains 990 µl of 100 mM Tris/ HCl buffer (pH 8.0, containing 2 mM $CaCl_2$, and 1M Phenyl acetate). The reaction is initiated after the addition of 10 µl of serum and the absorbance at 270 nm was continuously observed against the blank for 5 minutes at 25 °C in kinetic mode using a UV-visible spectrophotometer (Emcalb/ Germany). Enzymatic activity was presented as KU/L defined as the amount of the enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method.

Lactonase activity of PON1

This activity was measured following ŻAMOJĆ method (17). The assay started when 970 µl of 50 mM Tris/ HCl buffer (pH 8.0, containing, 1 mM of $CaCl_2$) and 10 µl of dihydrocoumarin (100 mM) as substrate were mixed with 20 µl of serum. The absorbance at 270 nm was continuously observed for 1 minute at 25 °C in kinetic mode using a UV-visible spectrophotometer (Emcalb/ Germany).and that the enzymatic activity of PON1 was calculated. Enzymatic activity was presented as KU/L defined as the amount of the enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method.

Total antioxidant status (TAS)

The TAS level was assessed using the Erel method (18), where a Fe^{2+}-o-dianisidine complex reacts with H_2O_2, generating hydroxyl radicals that reduce colorless o-dianisidine to a colored dianisidine radical. The reaction rate was monitored by tracking absorbance using PD-307 spectrophotometer (Apel/ Japan). Antioxidants in serum inhibit this oxidation and subsequent color formation, allowing for accurate TAS measurement. Results were expressed in mmol of ascorbic acid equivalent/L as standard. The procedure involved adding 25 µl of serum to the prepared solution, measuring initial absorbance at 444 nm, adding H_2O_2, and measuring final absorbance after 4 minutes at 444 nm.

Determination of Total Oxidant Status (TOS)

The TOS level was measured using the Erel method (19). This method involves the oxidation of Fe^{2+} to Fe^{3+} by oxidants under acidic conditions, forming a colored complex with xylenol orange. The color intensity is proportional to the oxidants in the sample. Glycerol enhances the reaction, and H_2O_2 is used for calibration. Results were expressed in µmol H_2O_2. The TOS level was measured using the Erel method (19). The color intensity is proportional to the oxidants in the sample. Glycerol enhances the reaction, and H_2O_2 is used for calibration. Results were expressed in µmol H_2O_2 Equiv/L. The process involves measuring the initial absorbance of a mixed solution of 105 µl of serum with xylenol orange solution, then $Fe\ (NH_4)_2(SO_4)_2.6H_2O$ was added, and the final absorbance was recorded at 560 nm after 4 minutes using PD-307 spectrophotometer (Apel/ Japan).

Determination of oxidative stress index (OSI)

The ratio of TOS to TAS yields the OSI, an indicator of the degree of oxidative stress:

OSI (arbitrary unit) = TOS (mmol H_2O_2 /L) \ TAS (mmol Vitamin C /L).

Statistical Analysis

Statistics were conducted using IBM SPSS Statistics 22. The qualitative data were represented as actual numerical values in an information table, and these data were presented as mean ± standard deviation (SD) for normally distributed data. The mean values were determined using an independent-

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samples t-test since the samples were collected independently. Additionally, a correlation test was conducted to examine the relationship between all mentioned variables through bivariate Pearson correlation. A probability value of 0.05 or less was considered a significant difference.

Results

The baseline information for each variable is summarized in the three tables presented below. The age distribution between patients and control groups was similar, with no significant difference. The FBG levels in the patients group were significantly higher than in the control group (P < 0.001). Urea levels also showed a significant elevation in patients (P = 0.006), while creatinine levels showed no significant difference. Additionally, a lower level of total protein was observed in the patients group, with significance (P= 0.013), as shown in Table 1.

Table 1. The characteristic profile of the patients and control groups.

Parameters	Control group Mean± SD	Type 1 DM group Mean± SD	P value
Age (year)	10.67 ± 4.04	9.91 ± 3.65	0.385
Gender (male/female)	(23/34)	(28/39)	-
Urea (mg/dL)	23.68 ± 4.23	28.21 ± 7.84	**0.006***
Creatinine (mg/dL)	0.61 ± 0.23	0.58 ± 0.20	0.346
Total protein (g/L)	71.36 ± 1.01	69.24 ± 1.12	**0.013***
FBG (mg/dL)	78.64 ± 16.75	201.66 ± 74.91	**<0.001***

Lipid profile analysis revealed that TC and HDL-C levels were significantly lower (P=0.012, P<0.001) in the patients group than the control. The TG and VLDL-C levels were significantly higher in patients, with P values less than 0.001 for both, while LDL-C showed non-significant difference, as shown in Table 2.

Table 2. Lipid profile of the patients and control groups.

Parameters	Control group	Type 1 DM group	P value
TC (mg/dL)	172.91 ± 46.15	148.82 ± 21.81	**0.012**
TG (mg/dL)	80.61 ± 26.75	106.97 ± 54.85	**<0.001**
HDL-C (mg/dL)	77.95 ± 17.95	51.15 ± 11.76	**<0.001**
VLDL-C (mg/dL)	16.01 ± 5.36	21.11 ± 4.86	**<0.001**
LDL-C (mg/dL)	76.36 ± 26.17	81.82 ± 31.41	0.850

Both enzymatic activities of PON1 and their specific activities showed significant reductions among patients (P < 0.001) in comparison to the control (Table 3). A reduction in the activity of the PON1 enzyme in T1DM and its specific activities were noticed with statistical significance across all data points in this table. The same results were noted for arylesterase / HDL-C, and lactonase /HDL-C ratios.

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Table 3. Paraoxonase activities of the patients and control groups.

Parameters	Control group	Type 1 DM group	P value
Arylesterase (KU/L)	4.54 ± 1.84	2.36 ± 1.17	**<0.001***
Lactonase (KU/L)	29.51 ± 9.92	21.9 ± 7.31	**<0.001***
Arylesterase /HDL-C	62.19 ±48.46	56.87±29.26	**0.034***
Lactonase /HDL-C	38.22 ±16.42	33.23±21.78	**0.008***
Arylesterase Specific activity	0.06±0.05	0.03 ± 0.01	**<0.001***
Lactonase Specific activity	1.98 ± 0.41	0.98 ± 0.31	**0.002***

Oxidative stress measurements indicated that TOS and OSI were significantly higher in patients compared to the control group (P< 0.001 and P= 0.001, respectively). Conversely, TAS was significantly lower in patients compared to the control group (P = 0.0036) (Table 4).

Table 4. The oxidative stress indices of the patients and control groups.

Parameters	Control group	Type 1 DM group	P value
TOS (mmol H_2O_2 /L)	28.37 ± 12.65	47.53 ± 25.57	**<0.001***
TAS (mmol Vitamin C /L)	0.40 ± 0.13	0.29 ± 0.18	**0.036***
OSI	84.23 ± 68.51	254.43 ± 117.6	**0.001***

Pearson correlation analysis revealed the presence of significant correlations between PON1 enzymatic activities and PON/HDL-C ratio, HDL-C level, and TAS level in the control group (Table 5). However, in the patients group, as seen in Table 6, most of these correlations were absent, except for that with PON1/ HDL-C ratio and HDL-C level.

Table 5. Correlation analysis of PON1 with the parameters in control groups.

Parameters	Arylesterase activity r (P value)	Lactonase activity r (P value)
Arylesterase /HDL-C	**0.784 (<0.001)***	0.523(0.09)*
Lactonase /HDL-C	0.598 (0.06)*	**0.687 (0.002)***
HDL-C	**0.420 (0.001)***	**0.396 (<0.001)***
TAS	**-0.359 (0.006)***	**-0.651 (0.003)***

Table 6. Correlation analysis of PON1 activities with the parameters in the T1DM group.

Parameters	Arylesterase activity r (P value)	Lactonase activity r (P value)
Arylesterase /HDL-C	**0.483 (<0.001)***	0.571(0.452)
Lactonase /HDL-C	0.666(0.219)	**0.328 (0.002)***
HDL-C	**0.379 (0.002)***	**0.366 (0.040)***
TAS	-0.022 (0.859)	-0.221 (0.732)

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Discussion

In the current study, total protein levels were significantly lower in the patients group compared to healthy controls, a finding consistent with previous research (20) which attributed this reduction mainly to a relationship between protein catabolism and elevated blood glucose levels. The FBG levels in the patients group were significantly higher than in the control group. Urea levels were significantly higher in patients, aligning with findings from another study (21) that suggested high blood sugar could cause dehydration. In contrast, no significant difference in creatinine levels was observed between the two groups. This finding is consistent with previous study that also reported no significant changes in creatinine, suggesting a potential disconnect between kidney function and urea levels (22). However, this contrasts with study on elderly individuals with diabetes, where reduced muscle mass resulted in lower creatinine levels (23).

Lipid profile analysis revealed that HDL-C levels were significantly lower in the patients group, a finding supported by previous research (24), which attributed this to the effects of increased oxidative stress or the utilization of this lipid type as an energy source due to insulin deficiency. A similar decrease in TC levels was observed, supporting the hypothesis that both of these lipid markers are impacted by the metabolic disturbances inherent to diabetes (24). On the other hand, TG and VLDL levels were significantly higher in the patients group, a result consistent with insulin resistance, while LDL-C levels showed no statistical significance, as noted in previous research (25, 26).

Oxidative stress markers also indicated a significant increase in free radicals among the patients group, along with a corresponding decrease in antioxidant levels. These findings align with prior studies that demonstrated a strong association between hyperglycemia and increased oxidative stress, alongside reduced antioxidant defenses (27, 28). Research suggests that elevated glucose levels increase electron production during mitochondrial glucose metabolism. These excess electrons interact with oxygen, resulting in the formation of reactive oxygen species (ROS), such as superoxide anions and hydrogen peroxide (29). The generation of ROS contributes to cellular damage and is linked to the development of diabetes-related complications, including cardiovascular disease, kidney damage, and neuropathy (30).

Regarding PON1, results showed a significant reduction in both arylesterase and lactonase activities in individuals with T1DM. This finding aligns with a previous study that reported decreased PON1 activity in diabetic individuals (31). Specifically, reduced PON1 activity impairs the body’s ability to detoxify harmful substances, including those produced by metabolic processes. The reduced hydrolytic ability for lactones in the patients group supports that the notion that antioxidant capacity of PON1 is compromised in the presence of elevated blood glucose. The relationship between PON1 activity and oxidative stress was also evident in this study, as reduced PON1 activities in T1DM patients coincided with increased oxidative damage. This aligns with numerous other studies showing weakened antioxidant defenses in diabetic patients, making them more susceptible to complications such as kidney and cardiovascular diseases (32, 33). Lower PON1 activity leads to lipid accumulation, contributing to atherosclerosis and increasing the risk of cardiovascular diseases (34, 35). Furthermore, the study confirmed a symbiotic relationship between PON1 and HDL, as indicated by Pearson correlation analysis, which revealed a significant positive correlation between PON1 activities (both arylesterase and lactonase) and HDL levels (Tables 4 and 5). This suggests that HDL is likely the primary source of the reduced enzyme activity. Elevated blood glucose

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levels in T1DM patients can oxidize HDL, a process facilitated by oxidative enzymes like NADPH oxidase, which generates free radicals that oxidize the lipids associated with HDL (36).

The decreased activity of arylesterase and lactonase due to high blood sugar levels and increased oxidative stress in diabetic patients leads to a diminished capacity to detoxify harmful substances and protect against oxidative damage, with increased accumulation of oxidative stress markers and lipid peroxidation products, further exacerbating cellular and tissue damage. This reduction in PON1 activity is associated with an increased risk of kidney, heart, and vascular diseases, highlighting the critical role of PON1 in maintaining oxidative balance and preventing disease progression in diabetic patients. Therefore, enhancing PON1 activity or mitigating oxidative stress could be potential therapeutic strategies to reduce the risk of diabetic complications and improve overall cardiovascular health in patients with diabetes.

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Conflicts of Interest

No conflicts of interest to declare.

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