Original Article

Paraoxonase 1 Activity is Associated with Interleukin-6 Levels in Type 2 Diabetes: Effects of Age and Gender

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Abstract

Background: A further understanding of the mechanisms linking inflammation to T2D and related complications can help prevent or control this silent but dangerous disease. This study was conducted to determine the association between paraoxonase 1 (PON1) activity and interleukin-6 (IL-6) in type 2 diabetes (T2D). Furthermore, we have evaluated the role of age and gender in the relationship between the PON1 activity and IL-6. **Methods:** A total of 105 people with T2D were enrolled in this study. IL-6 levels were determined using ELISA. For the PON1 activity assay, the hydrolysis rate of the substrate phenylacetate was spectrophotometrically assayed in serum at 270 nm. The determined velocities were the initial velocities of substrate hydrolysis. **Results:** PON1 activity was negatively correlated with IL-6 in total data ($r = -0.34$, $P = 0.001$). In both groups with age ≥50 and <50 years, PON1 activity was negatively correlated with IL-6, but the correlation was significant in patients aged 50 years and above ($r = -0.358$, $P = 0.005$) compared with patients with age <50 years. In both women and men, PON1 activity was negatively correlated with IL-6, but the correlation was significant in women ($r = -0.318$, $P = 0.006$) in comparison with men. **Conclusions:** Inverse association between PON1 activity and IL-6 in T2D may represent the oxidative–inflammatory interaction in this disease. Our findings highlight that at older ages and in women, the associations between lower PON1 activity and higher IL-6 concentrations are more evident, and this should be considered in patients with T2D.

Keywords: *Age, gender, interleukin-6, paraoxonase 1, type 2 diabetes*

Introduction

Type 2 diabetes (T2D) is a metabolic disease with complex pathophysiology, becoming a major health problem worldwide.[1] Its prevalence has been estimated to rise to 550 million by the year 2030 worldwide.[2] The prevalence of diabetes is increasing in Asian populations, including Iran.[3] Based on studies, chronic activation of proinflammatory pathways in target cells of insulin action may involve insulin resistance, obesity, and related metabolic disorders, including T2D.[4] Circulatory cytokines increase in metabolic syndrome and T2D as a result of inflammation.[5]

Paraoxonase 1 (PON1) is an esterase/lactonase that is tightly bound to high-density lipoprotein (HDL) particles in circulation.[6] This antioxidant enzyme can degrade oxidized phospholipids and hydrolyze lactones from lipoproteins, mainly low-density lipoprotein (LDL).[7] This enzyme was proposed to contribute to the anti-inflammatory activity of HDL by destroying biologically active lipids in mildly oxidized LDL (ox-LDL).[8] To improve the risk assessment of coronary artery disease, the PON1 activity has been proposed as an alternative to HDL-C in atherogenic lipid ratios.[9] PON1 plays an essential role in preventing or reducing cardiovascular complications in T2D patients through different mechanisms such as reducing ox-LDL concentrations, reducing macrophage ability uptake ox-LDL, and reducing foam cell formation.[10]

Interleukin (IL-6) is a 26-kDa protein generated by a wide variety of cells such as immune cells, endothelial cells, skeletal and smooth muscle cells, fibroblasts, astrocytes, and islet beta-cells.[11,12] IL-6 is a multifunctional and proinflammatory cytokine with key effects on immunoregulation and nonimmune events in most cell types and tissues.[12,13] Various studies have proposed the protective and

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pathogenetic actions of IL-6 in insulin resistance, beta-cell function, T1D, and T2D.[13] It was shown that chronically increased concentrations of specific inflammatory markers such as IL-6 and tumor necrosis factor (TNF)-α promote insulin resistance in skeletal muscle and endothelial dysfunction in the vasculature.[5]

Oxidant–antioxidant imbalance and immuno-related mechanisms can involve in the pathogenesis of T2D.[13,14] A further understanding of the mechanisms linking inflammation to diabetes and related complications can help to prevent or control this dangerous disease and its complications.[4] To the researchers' knowledge, no studies have yet been conducted to examine the association between PON1 activity and IL-6 in T2D in vivo. This study was conducted to determine the correlation between PON1 activity and IL-6 in T2D patients. Furthermore, we have evaluated the role of age and gender in the relationship between the PON1 activity and IL-6.

Methods

Study subjects

The cross-sectional study comprised 105 patients with T2D (age range 52.82 ± 10.58 years). This study was performed in the Northern Province of Iran, Mazandaran, Sari. The study was approved by the Ethical Committee at Mazandaran University of Medical Sciences (ethnic number: IR.MAZUMS.REC.96.3040), and informed consent was received from all participants. The participants underwent a physical examination and information, including medical history, medication, and personal habits, and were obtained using a questionnaire. All patients were diagnosed based on the diagnostic criteria as recommended by the WHO, incorporating both fasting glucose and a 2-h oral glucose tolerance test. All the patients were receiving metformin (1000 mg/day). The study's exclusion criteria were type 1 diabetes, insulin therapy, renal failure, chronic hepatic disease, autoimmune diseases, and malignancies.

Biochemical assays

Venous blood samples were collected from the participants after an overnight fast, and sera were isolated by low-speed centrifugation, and their aliquots were stored at −70°C. The samples were assayed for IL-6 by ELISA method using the kit from eBioscience, USA (the inter-assay and intra-assay of coefficients of variation were 5.5% and 3%, respectively). Serum fasting glucose, HDL-C, and LDL-C levels were determined using commercially available kits (Pars Azmoon, Tehran, Iran).

For the PON1 activity assay, the hydrolysis rate of the substrate phenylacetate was spectrophotometrically assayed in serum using a double-beam spectrophotometer (UV 1800, Shimadzu, Japan) at 270 nm. PON1 activity was analyzed by the addition of serum to Tris/HCL buffer (0.1 M, pH 8.0) containing phenylacetate (1 mM) and $CaCl_2$ (1 mM). The determined velocities were the initial velocities of substrate hydrolysis. The molar extinction coefficient was 1310 M^{-1} cm^{-1}. One unit of PON1 activity is defined as 1 μmol phenol formed per minute.

Statistical analyses

The distributions of all variables were tested using the Lilliefors test. The sample size was calculated using PASS software (version 11, UT, USA). Spearman's correlation coefficient was used to analyze the association between the study parameters. Statistical significance was accepted at P less than 0.05. Statistical analyses were conducted using the software SPSS (version 16.0).

Results

The age and biochemical characteristics of the study subjects are shown in Table 1. The mean values of fasting glucose, PON1 activity, and IL-6 levels were 146.04 (mg/dL), 72.78 (μmol/min/mL), and 7.96 (pg/mL), respectively.

Our findings indicated that PON1 activity was negatively correlated with IL-6 in total data (r = −0.34, P = 0.001), as shown in Figure 1. Further analyses according to age showed that in both groups with age ≥50 and <50 years, PON1 activity was negatively correlated with IL-6, but the correlation was significant in patients aged 50 years and above (r = −0.358, P = 0.005) compared with patients with age <50 years (r = −0.322, P = 0.067) [Figure 2].

In addition, when correlation analyses were performed according to gender [Figure 3], our findings indicated that both in women and men, PON1 activity was negatively correlated with IL-6, but the correlation was significant in women (r = −0.318, P = 0.006) in comparison with men (r = −0.432, P = 0.065).

Discussion

According to our results, PON1 activity was negatively correlated with IL-6 in T2D patients. The analyses according to gender and age demonstrated that these important factors influence the associations between PON1 activity and IL-6, representing an interaction between oxidative and inflammatory processes in T2D.

Table 1: Age and biochemical characteristics of the study subjects (*n*=105)

Parameter	Mean ± SD
Age (years)	52.82 ± 10.58
Fasting glucose (mg/dL)	146.04 ± 28.69
HDL-C (mg/dL)	46.4 ± 16.1
HDL-C (mg/dL)	103.4 ± 35.6
PON1 activity (μmol/min/mL)	72.78 ± 19.75
IL-6 (pg/mL)	7.96 ± 4.16

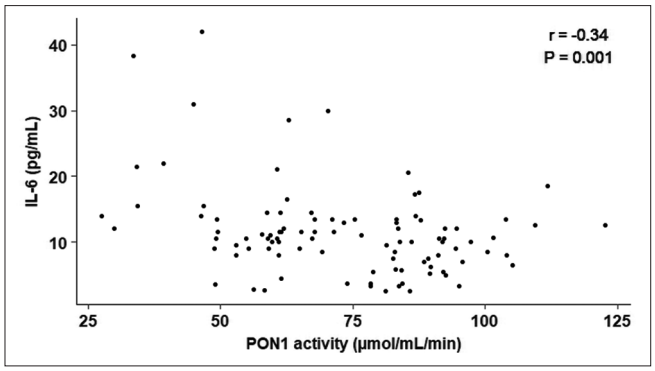

Figure 1: Correlation between paraoxonase 1 (PON1) activity and interleukin-6 (IL-6) in the study subjects

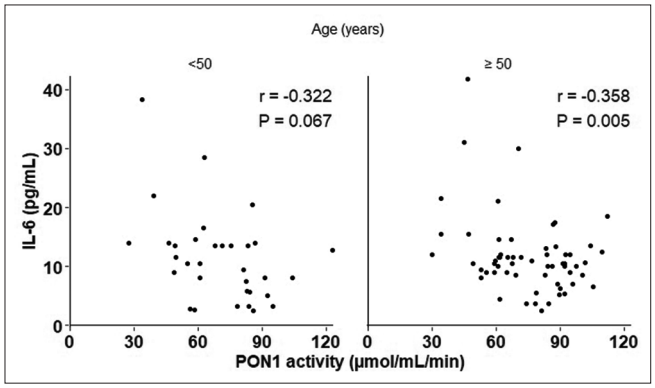

Figure 2: Correlations between paraoxonase 1 (PON1) activity and interleukin-6 (IL-6) in the study subjects according to age

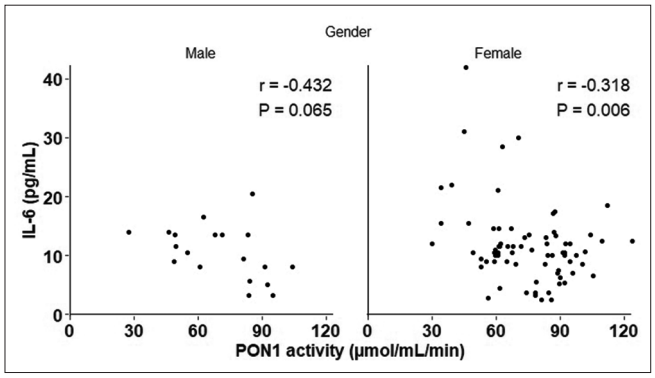

Figure 3: Correlations between paraoxonase 1 (PON1) activity and interleukin-6 (IL-6) in the study subjects according to gender

It was indicated that the systemic concentrations of IL-6 are increased in T2D patients, and this cytokine was implicated in T2D development.[11,15] On the other hand, PON1 activity is inversely related to inflammatory responses.[8] The role of PON1 in diabetes mellitus has been expressed in several studies.[16] PON1 plays a protective role in diabetes development, attributed to its anti-oxidative characteristics.[14] The observations reported by Brinholi *et al*.[17] in accordance with our study showed an association between the decreased activity of PON1 and increased cytokine concentrations, including IL-6, IL-4, and IL-10 in patients with drug-naïve first episode psychosis. It was reported that IL-6 directly decreases the PON1 activity in a dose-dependent manner.[18] It was also shown that cytokines such as IL-6 reduce the expression of apoA-I and PON-1 by inhibiting peroxisome proliferator-activated receptor-α activation.[19] Our results further supported by Aharoni *et al*.[8] reported that recombinant PON1 could significantly reduce the spontaneous secretion of TNF-α and IL-6 from macrophages in a dose-dependent manner.

According to Van Lenten *et al*.,[20] IL-6 may contribute to short-term PON regulation but not long-term regulation. This may be one of the reasons that in the chronic situation, this enzyme activity was reduced despite the absence of IL-6. It should be noted that the short-term regulation of PON activity mediated by IL-6 might worsen the inflammatory reaction in atherosclerosis. Besides, inflammatory cytokines may mediate the impacts of oxidized phospholipids in acute oxidative stress. The oxidized phospholipids can enhance IL-6 concentrations and inversely lead to decreased PON1 levels. These phospholipids increase in T2D.[20] This issue seems to be confirmed in our study by the inverse association between PON1 activity and IL-6.

Based on our results, the correlations were statistically different when the analyses were performed according to age and gender. Only in the group age ≥50 years, there was a significant correlation between PON1 activity and IL-6. Also, the correlation between these factors was significant in women, not men. The findings indicate that age and gender may have affected the correlations between the PON1 activity and IL-6.

The present findings are supported by previous studies, which showed IL-6 levels elevate with age,[21] and inversely, PON1 is reduced with increasing age.[22] Reduced PON1 activity with age may be due to the elevated susceptibility of LDL oxidation and increased oxidative stress, influencing the enhanced incidence of atherosclerosis with age.[23] Based on Albani *et al*.,[21] circulating blood concentrations of IL-6 elevate with age, and there is a significant positive correlation between age and IL-6 levels. Age was also associated with increased lipid peroxidation markers in circulation, impairing PON1 activity.[24] Besides, women have a more significant inflammatory risk profile than men due to sex differences in proinflammatory cytokines such as IL-6. It was reported that women have higher levels of IL-6 as compared to men, which can be related to greater monocyte expression of IL-6 across the circadian period in women.[25]

The small sample size should be considered a limitation of the current study, and so the authors recommend large prospective studies on this subject to help validate these findings. Moreover, unfortunately, we did not have access to data on the duration of diabetes (it may be related to IL-6 levels and PON1 activity).

Conclusions

Inverse association between PON1 activity and IL-6 levels in T2D may represent the oxidative–inflammatory interaction in this disease. Our findings highlight that at older ages and in women, the associations between lower PON1 activity and higher IL-6 concentrations are more evident, which should be noted in patients with T2D.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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