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Original Article

Evaluation of paraoxonase-1 activity in Yemeni Type 2 diabetes mellitus and its association with atherogenic risk factors

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Abstract

Objective: To evaluate the Paraoxonase-1 (PON1) activity in Yemeni patients with Type 2 DM and examine its association with the atherogenic risk factors.
Methods: This was a case-control study carried out on 97 subjects: 46 healthy subjects from both genders (control) and 51 Type 2 DM patients. Biochemical parameters include PON1, fasting blood glucose (FBG), glycated hemoglobin (HbA1c), and lipid profile were analyzed.
Results: Serum PON1 was significantly (p = 0.001) lower in Type 2 DM patients by 10.1% with respect to the healthy control subjects. Moreover, FBG, HbA1c and total cholesterol were significantly higher in Type 2 DM patients as compared to the healthy controls. Serum PON1 activity was positively correlated with high-density lipoprotein cholesterol – HDL-c (r = 0.383, p = 0.006) and inversely correlated with HbA1c (r = - 0.341, p = 0.014).
Conclusion: Paraoxonase-1 was lower in Type 2 DM patients and being positively correlated with HDL-c and inversely correlated with HbA1c.

Key wards: Paraoxonase-1, Type 2 Diabetes Mellitus, Atherogenic risk factors

Introduction:

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, and or both. The chronic hyperglycemia of diabetes is associated with long-term damage, failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. Type 2 DM is caused by insulin resistance, inadequate secretion of insulin, is generally associated with overweight and obesity [2].

The paraoxonase (PON) enzyme family is constituted by three members: PON1, PON2, and PON3. Paraoxonase-1 (PON1) is the most studied member of the paraoxonase family and is classified as an aryldialkylphosphatase [3]. Paraoxonase-1 is a 44 kD calcium-dependent glycoprotein synthesized by the liver and associated with high-density lipoprotein (HDL) contributes to the lipoprotein functionality [4-6]. It has been widely demonstrated that PON1 protects HDL, low-density lipoprotein (LDL), endothelial cells, and intimal macrophages from oxidative insult [3,7]. This protective action occurs via hydrolysis of the potential physiological substrate, lipo-lactones, which originate from the oxidative damage of phospholipids present on cell and lipoprotein surfaces [8]. Low PON1 activity has been reported to increase the development of atherosclerosis [9].

Nonetheless, the evidence of differential expression of PON1 in the context of Type 2 DM is still unclear. The recognized evidence about PON1 expression in Type 2 DM is related to its genetic polymorphisms, which have

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been suggested to be responsible for variations in serum PON1 activity of Type 2 DM patients [10]. Consistently, the majority of population-based studies on the PON1 phenotype found that low enzymatic activity is associated with Type 2 DM and related clinical complications, and this, in turn, could be due to the increased levels of pro-atherogenic oxidized LDL and HDL of these patients [11,12].

In view of the fact that PON1 levels and activity are significantly impaired in individuals with cardiovascular and liver diseases, as well as in Type 2 DM and obesity [13]; the aim of this study, therefore, was to evaluate the paraoxonase-1 (PON1) activity in Yemeni patients with Type 2 DM and examine its association with the atherogenic risk factors.

Subjects and Methods

This study was a case-control study that was carried out on 97 subjects aged 30-60 years: 46 were apparently healthy individuals with no history of diseases such as hypertension, diabetes, inflammation, tumors, and cardiovascular disease serving as control group and 51 Type 2 DM patients attending the outpatient Clinic of the National Diabetic Centre in Al-Thawra Hospital, Sana'a – Yemen. All the Type 2 DM patients were diagnosed more than 2 years and controlled by diet or by oral anti-diabetic agents. Patients with immunosuppression treatment, renal disease, or acute illness at the time of testing were excluded from this study. The study protocol was approved by the Institutional review board (IRB) of the Faculty of Medicine and Health Sciences, Sana'a University. Informed consent form was obtained from all individuals after explaining the purpose and nature of the study.

The patients' height and weight were measured and body mass index (BMI), defined as weight (kg)/ height squared (m2), was calculated. Waist circumference was measured halfway between the lower rib margin and the anterior superior iliac spine. Blood pressure (BP) measurements were taken from each patient's right arm in the seated position by using an Omron IntelliSense Automatic Blood Pressure Monitor after 10 min of rest. Two to three successive BP readings were obtained at 5-minute intervals and averaged. Standardized questionnaire(s) was administered to collect participants' demographic and clinical data. Venous blood (5 ml) was collected from each individual after an overnight fast of more than 10 hours and divided into two vacuumed tubes; 4 ml into plain tubes for biochemical assay and 1 ml into a K2EDTA tube for HbA1c. The serum from each sample was separated within 30 minutes and aliquoted into 2 Eppendorfs and immediately stored at -20ºC for biochemical analysis. Haemolysate were prepared immediately for HbA1c determination within 2 hours of blood collection.

Biochemical Analysis

Fasting blood glucose (FBG), triglyceride (TG), total cholesterol, HDL-cholesterol (HDL-c), and LDL-cholesterol (LDL-c) were measured on an automated analyzer, the Cobas Integra 400/400 (Roche Diagnostic, USA), using the respective Roche Diagnostic kits. Glycated haemoglobin (HbA1c) was measured on the Cobas Integra 400/400 (Roche Diagnostic, USA) using a turbidimetric inhibition immunoassay. The Paraoxonase-1 activity was measured on Jenway 6405Uv/Vis spectrophotometer using ZeptoMetrix kits (Buffal, New York).

Statistical analysis

The statistical analyses were performed on Social Package of Social Sciences (SPSS) version 10 (SPSS Inc, Chicago, IL, USA). Results were expressed as median (25th and 27th percentiles) and the significance was analyzed by independent t-test when comparing two groups. Pearson correlation was used for the association between Paraoxonase-1 and the atherogenic risk factors. The significant difference was indicated if p value was < 0.05.

Results

The results presented in Table 1 shows the demographic and biochemical parameters in Type 2 DM patients. Of the

metabolic syndrome factors cholesterol and LDL-c were significantly higher (p = 0.01 and 0.008) in Type 2 DM patients by 12% and 20.9% respectively as compared with the control group, whereas HDL-c was significantly (p = 0.04) lower by 14.9%. However, triglyceride levels were non-significantly higher in Type 2 DM patients by 20.2%. The diabetic parameters FBG and HbA1c were significantly (p = 0.001) higher in Type 2 DM patients than control group by 2.3 folds and 65.6% respectively. Serum paraoxonase-1 was significantly (p = 0.001) lower in Type 2 DM patients by 10.1% with respect to control group.

Table 1: Demographic and biochemical parameters of Type 2 DM patients and control subjects

	Control (n = 46)	Type 2 DM (n = 51)	p-value
Age (years)	52 (45 - 60.5)	51 (45 - 60)	0.951
BMI (kg/m^2)	25 (22.9 - 31.4)	27 (24.4 - 31.1)	0.275
Waist Circumference (cm)	96.2 (91 - 104)	91 (83.2 - 103.4)	0.080
SBP (mmHg)	110 (108 - 120)	120 (100 - 120)	0.605
DBP (mmHg)	80 (70 - 80)	75 (70 - 80)	0.311
Triglyceride (mg/dl)	125.6 (88.6 - 182.6)	151 (105.6 241.7)	0.103
Cholesterol (mg/dl)	186.5 (147.1 - 225.9)	208.8 (160.9 - 256.7)	**0.01**
HDL-c (mg/dl)	45.8 (35.4 - 54)	39 (31.37 - 50.3)	**0.04**
LDL-c (mg/dl)	105.9 (90.4 - 138.9)	128 (101 - 184.5)	**0.008**
FBG (mg/dl)	81.72 (77.4 - 93.6)	188 (150.1 - 224.5)	**0.001**
HbA1c (%)	6.04 (5.8 - 6.5)	10 (8.1 - 12.4)	**0.001**
Paraoxonase1 (IU/l)	47.4 (44 - 51)	42.6 (35.9 - 49.3)	**0.001**

Data are expressed as median (25th and 27th percentiles)

Table 2 shows the Pearson correlation between paraoxonase-1 and both metabolic syndrome factors and diabetic parameters in Type 2 DM patients. Of all the parameters tested, paraoxonase-1 was positively correlated with HDL-c (r = 0.383, p = 0.006) and negatively correlated with HbA1c (r = 0.341, p = 0.014)

Table 2: Pearson correlation between paraoxonase-1 and atherogenic risk factors

	R	*p-value*
Age	0.04	0.766
BMI	- 0.104	0.467
Waist Circumference	- 0.123	0.389
SBP	- 0.219	0.122
DBP	- 0.094	0.512
Triglyceride	- 0.028	0.846
Cholesterol	0.112	0.434
HDL-c	**0.383**	**0.006**
LDL-c	0.054	0.705
FBG	- 0.110	0.433
HbA1c	**- 0.341**	**0.014**

Discussion

The results presented in this study shows PON1 to be significantly lower in Type 2 DM patients with respect to the control subjects which is in agreement with several studies [14-17]. The decrease in PON1 activity could be attributed to the prolong hyperglycemia in patients with diabetes due to impairment of insulin pathway leading to oxidative stress (atherogenesis) and the subsequent induction of antioxidant mechanisms as a protective measure. Moreover, hyperglycemia causes glycosylation of proteins including enzymes and this process may lead to the observed decrease in PON1 activity, especially due to glycation of HDL-PON1 [11,18]. The decrease in PON1 enzyme activity has been reported to reduce the ability of the body to normalize lipid-peroxidation, which thereby accelerates the oxidative stress. Moreover, the concentration of PON1 enzyme was reported to be lower in Type 1 DM than Type 2 DM and attributed it to the duration of diabetes in Type 1 DM which is usually longer than Type 2 DM as well as the complete absence of insulin that leading to prolong glycation of the enzyme protein [19].

Our results also highlight the dyslipidemia which is closely associated with Type 2 DM [20], with HDL-c being lower in the diabetic patients. HDL-cholesterol is known to affect glucose metabolism, through the alteration of glucose uptake in skeletal muscle and the promotion of insulin release by β-cells [21]. Moreover, our results show PON1 activity to be positively correlated with HDL-c and inversely correlated with HbA1c. This further constitute supportive evidence to the idea that that PON1 plays a role in the protection against lipid peroxidation and being associated with the more atherogenic profile in Type 2 DM patients [22]. It is well known that PON1 enzyme decreases accumulation of oxidized lipids from LDL because of its ability to decrease hydroperoxides as well as HDL that reduce the accumulation of lipid peroxides in LDL [23]. Type 2 DM was associated with decreased HDL lipid and increased LDL, therefore the susceptibility to cardiovascular diseases (CVD) risk is about two to four fold higher in DM compared to healthy people [24].

Conclusion

The present study shows that PON1 activity was lower in Type 2 DM patients, and being positively correlated with HDL-c and inversely correlated with HbA1c.

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