Paraoxonase-1 (PON1) activity in patients with coronary artery diseases and in diabetic patients

Étude de l’activité de la paraoxonase-1 (PON1) chez des patients coronariens et des patients diabétiques

Khadija Amine1
Abdelouahed Atouk1
Samya Moussamih2
Rachid Saile1
Afaf Mikou3
Anass Kettani1

1 Laboratoire de recherche sur les lipoprotéines et l’athérosclérose, Unité de recherche associée au CNRST-URAC 34-Université Hassan II Mohammedia, Faculté des sciences Ben M’lk, Casablanca, Maroc
<akettani9@yahoo.fr>
2 Laboratoire de microbiologie, pharmacologie, biotechnologie et environnement, Faculté des sciences Ain Chock, Casablanca, Maroc
3 Equipe physico chimie et spectrométrie appliquées à l’environnement, Faculté des sciences Ain Chock, Casablanca, Maroc

Abstract. Cardiovascular diseases are the main cause of mortality in the world, diabetics and patients with coronary artery diseases in particular. In fact, the increase of cardiovascular risk was established in many epidemiological and clinical studies. The aim of this work is to study both the lipid profile and the enzymatic activity of PON1 in diabetics and coronary patients from Morocco (Casablanca region) along with the cardiovascular risk factors in this population. Three groups of Moroccan subjects were investigated: 36 patients with coronary artery diseases, 110 diabetic patients and 100 healthy subjects (control group). Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (c-HDL) levels were evaluated using colorimetric methods. Low-density lipoprotein cholesterol (c-LDL) was calculated according to the Friedewald’s formula. Serum activity of PON1 was measured by spectrophotometry. Compared to healthy subjects, we noted a significant decrease of PON1 activity in coronary artery disease (285 U/mL ± 180 U/mL; P < 0.05) and in diabetic (167 U/mL ± 71 U/mL; P < 0.05) patients. In addition, we found that diabetic patients recorded significantly elevated LDL, TG and TC levels. In parallel, coronary artery disease patients scored TG level. The present study revealed an abnormal lipoprotein profile associated with hypertriglyceridemia, low levels of c-HDL, high levels of c-LDL and significant decrease of PON1 activity. These findings confirm the high risk of cardiovascular diseases in diabetic and coronary artery disease patients.

Key words: paraoxanase-1, cardiovascular diseases, coronary artery diseases, type II diabetes

Résumé. Les maladies cardiovasculaires représentent la principale cause de mortalité dans le monde, en particulier chez les patients diabétiques et coronariens. En fait, l’augmentation du risque cardiovasculaire a été montrée par de nombreuses études épidémiologiques et cliniques. Le but de cette étude est de déterminer à la fois le profil lipidique et l’activité enzymatique de la paraoxonase-1 (PON1) chez des patients diabétiques et les coronariens marocains et d’évaluer les facteurs de risque qui seraient en faveur de l’atteinte cardiovasculaire dans la population étudiée. Trois groupes de sujets marocains ont été étudiés : 36 patients coronariens, 110 diabétiques et 100 sujets sains (groupe témoin). Le cholestérol total, les triglycérides et le cholestérol des lipoprotéines de haute densité ont été évalués en utilisant des méthodes colorimétriques. Le cholestérol des lipoprotéines de faible densité a été calculé selon la formule de Friedewald. Les activités sériques de PON1 ont été mesurées par spectrophotométrie. Par rapport aux sujets sains, nous avons noté une diminution significative de l’activité de PON1 chez les coronariens (285 U/mL ± 180 U/mL ; p < 0.05) et chez les diabétiques (167 U/mL ± 71 U/mL ; p < 0.05). En outre, nous avons constaté que les patients diabétiques enregistrent une élévation significative des concentrations de...
Cardiovascular disease (CVD) is the leading cause of death and morbidity in the world, especially in patients with type II diabetes and coronary artery diseases (CAD). At the national level and according to the report of the World Health Organization, 60,375 Moroccans die from CVD each year. Increased left ventricular mass, plaque rupture, thrombus formation, vasospasm have all been associated with heightened cardiovascular response to acute stress. It has been argued that heightened cardiovascular reactivity to stress contributes to the development of future CVD [1]. Patients with type II diabetes mellitus (DM) and coronary heart disease (CHD) have a high mortality risk related to CVD [2]. Dyslipidemia with both quantitative and qualitative aspects or characteristics of DM and coronary diseases are among the major causes of these two pathologies [3]. The risk of developing a DM is partly attributed to an increased prevalence of classic CHD risk factors, hyperglycemia and a highly atherogenic lipid profile. Despite that, the CVD are multifactor disorders where environmental, dietary habits and lifestyle play an important role as risk factors, the prevalence of CVD is increasing in less urbanized, developed populations across the world, as their lifestyles change to a so-called “western style”, with increasing consumption of dietary saturated fat, cholesterol and salt, cigarette smoking, decreased physical activity and the rise in CVD risk factors including obesity and diabetes. Other known factors that contribute to CVD risk are stress and high alcohol intake. Among all these factors, hypercholesterolemia is the leading cause of death from CVD, especially high cholesterol related to low-density lipoprotein (LDL) [4]. Another important factor is the cholesterol transported by high-density lipoprotein (HDL). Recent studies have attributed the antioxidant properties to HDL and the amount of fat produced from oxidized LDL is indeed clearly reduced in the presence of HDL [5]. These observations suggest the presence of enzymatic mechanisms within the HDL that is able to remove oxidized lipids. The paraoxonase-1 (PON1; aryldialkylphosphatase, EC 3.1.8.1), the main HDL enzyme, would be responsible for this antioxidant property. Results of Mackness et al. have demonstrated that PON1 could prevent oxidation of LDL [6]. These data have confirmed the PON1 action on oxidized lipids and the effect of this enzyme on CVD. Serum PONs are able to decrease the risk of coronary artery disease (CAD) by destroying proinflammatory molecules involved in the initiation and progression of atherosclerotic lesions [7]. PONs are a series of serum esterase enzymes synthesized in the liver. PON1 is characterized by its ability to hydrolyze the organophosphate substrate paraoxon, which is the toxic metabolite of the insecticide parathion. It belongs to the family of serum PONs, including PON1, PON2 and PON3. PON1 and PON3 are secreted from the liver into the blood circulation and are associated with HDL particles [8]. Rosenblat et al. have recently described the role of PON1 hydrolytic activity to mediate inhibition of LDL oxidation and stimulation of cholesterol efflux from macrophages. The authors also reported that apolipoprotein (Apo) A-I in HDL stimulates PON1 lactonase activity [9]. Furthermore, Mackness et al. demonstrated that PON1 could prevent accumulation of lipoperoxides in LDL [6]. PON1 is implicated in preventing atherogenesis and CHD. However, there have been only a few studies assessing serum concentrations of PON1 within CHD [10, 11]. PON1 is involved in numerous diseases. Regarding the role of PON1 in renal diseases, a Moroccan study showed that PON1 activity was decreased in haemodialysis patients, especially in elderly ones [12]. In our laboratory, other studies have investigated the effect of the argan oil on the activity of PON1 in patients with CVD, and the analysis of the results shows that PON1 activities increase significantly [13]. To our knowledge, the role of PON1, particularly in diabetes and CAD, has not been yet established in Morocco. In the present work, we studied the lipid profile, along with the serum PON1 activity. We assess some cardiovascular risk factors in diabetic and CAD patients during treatment and we compared these findings with those in healthy controls.

Material and methods

Study participants

Two groups of patients including 36 CAD patients from cardiology service and 110 diabetic patients from endocrinology service in Caisse nationale de Sécurité
sociale (CNSS) hospitals (Casablanca, Morocco). The measurements of both PON1 activity and lipid parameters of patients with coronary pathologies were performed in advanced stages of CAD (both the clinical events and angiographic evidence). The average duration of diseases (or a disease if single) between the first vascular accident and hospitalization is 122 ± 94 months. Fifty percent of diabetic patients were under anti-diabetic medication. We noted that 18 of them take a medication in the biguanide family (metformine), and 24 had a medication in the hypoglycaemic sulfamide family (benclamide, diamicron, amarel), and eight take insulin. Also, we noted a normal creatinine level (60 μmol/L) and a normal glomerular filtration rate hence the absence of any renal failure and any diabetic nephropathy. One hundred healthy subjects matched for age and gender served as controls. The control group had a normal lipid profile, an absence of cardiovascular risk factors and no CVD-related pathologies. All patients gave informed consent and the study was approved by the local ethics committee. The main clinical characteristics of all study groups are summarized in table 1.

Lipid levels determination
A blood sample was collected during the clinical visits, by venipuncture (5 mL of venous blood) after a 12-hour overnight fast. Lipid measurements were processed immediately and the remaining serum was stored at -20°C. Serum total cholesterol (TC) and triglyceride (TG) levels were measured by routine enzymatic methods (Biosytems, Spain). Serum HDL cholesterol (c-HDL) was enzymatically determined by separating HDL from plasma by precipitation of the (LDL + VLDL (Very Low Density Lipoprotein) fraction with a phosphotungstic acid-magnesium chloride solution (Biosytems, Spain). Serum LDL cholesterol (c-LDL) was calculated according to the Friedewald’s formula [14].

Paraoxonase-1 activity
PON1 activity was measured after the reaction of paraoxon (O,O-diethyl-O-p-nitrophenylphosphate) hydrolysis into 4-nitrophenol catalysed by the enzyme. The generation of 4-nitrophenol was followed spectrophotometrically: 50 μL serum was dissolved in 1 mL Tris-HCl buffer (100 mmol/L, pH 8.0) containing 2 mmol/L CaCl₂ and 5.5 mmol/L paraoxon. We measured the absorbance at 412 nm (25°C) using a Helios UV-Visible Spectrophotometer. Enzyme activity was calculated using the molar extinction coefficient 17,100 Mcm⁻¹. One unit of PON1 activity is defined as 1 nmol of 4-nitrophenol formed per minute (U/min) under the assay conditions mentioned above.

Statistical analysis
Data were summarized as the mean of ± S.D. Variables were compared by Student’s t-test. Analysis of variance post-hoc test was performed for comparison among the three groups. Correlation coefficients between all parameters studied were calculated by Pearson’s correlation analysis using the XLSTAT 7.5 program whose operation is based on Microsoft Excel for data entry and results publication. P values less than 0.05 were considered statistically significant.

Table 1. Main clinical characteristics and parameters studied for the diabetic and coronary patients enrolled in this study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAD patients</th>
<th>Diabetics patients</th>
<th>Control subjects</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), mean ± S.D.</td>
<td>62 ± 10</td>
<td>58.30 ± 10</td>
<td>59 ± 12.16</td>
<td>-</td>
</tr>
<tr>
<td>Gender (male/female), n</td>
<td>15/21</td>
<td>52/58</td>
<td>50/50</td>
<td>-</td>
</tr>
<tr>
<td>Glycemia (mmol/L)</td>
<td>7.15 ± 0.20</td>
<td>8.03 ± 3.35</td>
<td>4.90 ± 4.70</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30 ± 2.6</td>
<td>29 ± 2.2</td>
<td>20.5 ± 1.5</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Treatments</td>
<td>Statins</td>
<td>Type 2 medication</td>
<td>None</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.88 ± 1.43</td>
<td>5.16 ± 1.10</td>
<td>4.41 ± 0.89</td>
<td>&lt;0.01* NS**</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.86 ± 1.16</td>
<td>1.48 ± 0.90</td>
<td>1.14 ± 0.42</td>
<td>&lt;0.01* NS**</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.03 ± 0.42</td>
<td>0.49 ± 0.30</td>
<td>1 ± 0.25</td>
<td>NS* &lt;0.01**</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.52 ± 1.29</td>
<td>4.64 ± 1</td>
<td>2.94 ± 0.63</td>
<td>NS* &lt;0.01**</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: not significant; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein. *Signification between control subjects and coronary patients; CAD: coronary artery disease. **Signification between control subjects and diabetic patients.
Results

Lipid profile in coronary and diabetic patients
Analyses of lipid parameters were carried out on fresh sera. Frozen specimens were used for other measurements in the same period. The variation of parameters studied in both CAD patients and diabetic patients is shown in figure 1, comparing to the controls. A significant increase in TG in CAD patients, the other parameters did not vary significantly in the same group. However, in diabetic patients, we noted a significant decrease in c-HDL, and a significant increase in c-LDL, TC and TG levels compared to specimens from control subjects.

Evaluation of the cardiovascular risk factors among diabetic and CAD patients
In this study, we evaluated some cardiovascular risk factors in patients with diabetes and with coronary diseases according to the variation of lipid parameters and PON1 activity. Two hundred questionnaire copies have been distributed and analyzed as shown in table 2. The questionnaires explored nine variables: gender, age, body mass index (BMI), physical inactivity, family history, smoking, alcohol intake, hypertension and diabetes. For dyslipidemia factor, serum lipid levels (c-HDL, c-LDL, TG, TC) are reported in figure 1.

Paraoxonase-1 activity in coronary and diabetic patients
In addition, figure 2 indicates that both CAD patients and diabetic patients show a significant decrease in PON1 activity respectively (285 U/mL ± 180 U/mL; $P < 0.05$) and (167 U/mL ± 71 U/mL; $P < 0.05$) in comparison with the control subjects.

Discussion
In the present study, we analyzed the lipid profile, the PON1 activity, along with the cardiovascular risk in both CAD patients and diabetic Moroccan patients. CAD patients who suffer from cardiac pathologies (myocardial infarction, vascular disease) for about 11 years showed a relatively normal lipid profile except for TG level. The rate of TG on coronary patients showed a significant increase (1.63 ± 0.01), and the TC showed a slight increase (1.89 ± 1.5) compared to the control group. However, no significant difference was observed in c-HDL and c-LDL between the coronary and the control groups (figure 1). Participants with CAD had higher BMI (31 ± 1.89). They also had almost 50% prevalence of family history. Hypertriglyceridemia is known to be related to a decrease of lipolytic enzymes activity, and may be a consequence of the increase of TG level. Elevated TG and TC levels were associated with increased prevalence of other coronary risk factors, including DM, hypertension, and elevated TC [15]. The convergence of a variety of cardiovascular risk factors within population with CAD may explain the hypertriglyceridemia and hypercholesterolemia. As mentioned in table 2, obesity, sedentary lifestyle, hypertension, family history and diabetes affection are key risk factors for CVD in this population. Although CVD mortality was trending downward for almost 50 years, resurgence, both nationally and globally, has occurred. Diet and lifestyle increase CVD risk both directly and indirectly. Direct effects include biological, molecular, and physiologic alterations, including inflammatory stimuli and oxidative stresses. Indirect effects include diabetes, dyslipidemia and hypertension [16]. In addition, the absence of statistical significance of c-HDL and c-LDL levels may be due to the nature of treatment.
followed by the patients at the enrolment time such as the statin (regular dose of simvastatin), which establish the normal concentrations of lipid parameters. The statin has demonstrated a spectacular profile in primary or secondary prevention of cardiovascular illneses by the improvement of the lipid profile [17]. PON1 activity was significantly lower (285 U/mL ± 180 U/mL; \( P < 0.05 \)) among participants with CAD compared to the control group. These results were supported by other studies [18, 19] and showed that PON1 activity decreases significantly in coronary subjects. The PON1 activity has been shown to be lower after acute myocardial infarction [20]. It is also lower in patients with familial hypercholesterolemia and DM, who are more prone to CAD [21]. This has led to the hypothesis that the lower the PON1 activity is the higher will be the accumulation of oxidized LDL and risk of CAD. PON1 gene polymorphisms may influence variability of the enzyme activity and some cross-sectional, in fact case-control studies have described an association between CVD or cardiovascular events and PON1 gene polymorphism in coronary subjects [22]. These findings agree with our results concerning the coronary subjects. Serum PON1 activity greatly varies among individuals and populations due to the PON1 genetic polymorphism, but also to the gene environment [23]. Factors influencing serum levels of PON1, either genetic or environmental as demonstrated earlier, will in turn affect the capacity of HDL to protect LDL from oxidation and, consequently, may be linked to atherosclerosis [24]. Some studies have shown that PON1 levels and activities were independent of PON1 polymorphism in patients with CAD. PON1 has also been identified as an independent, genetic risk factor for vascular disease, particularly in coronary patients [25, 26]. The coronary patients presented no abnormalities in the level of the lipid profile except for TG levels, the decrease of PON1 activity could be explained genetically. The coronary patients may possess the phenotype A and this can explain the lower activity within this population.

In diabetic population, we observed a decrease of c-HDL level, and an increase of c-LDL, TG and TC levels. These results were supported by several studies [27] and these findings may be associated with high risk of CVD development in diabetic patients. Several studies showed that DM is itself a risk factor for several CVD, whose majority are descended from atherosclerosis [28]. Results of Lehto et al. [29] showed that the lipid abnormalities could play a major role in the occurrence of the cardiovascular accidents in diabetic patients. Effectively, the most prevalent diabetic cardiovascular risk factors in diabetic participants were dyslipidemia, it affected all lipid parameters, diabetes, family history and the development of an overweight. All these factors will favor the installation of CVD in diabetic patients and decreased activity of PON1. To assess the control of glycemia in the 110 diabetic patients, the average value was 7.5 ± 0.2% of total glycated haemoglobin. This value is in good agreement with the average blood glucose of diabetic patients to consider that diabetes is slightly unbalanced. In addition, only half of these patients are treated with sulfamides, biguanides and insulin drugs hence the insufficiency of the treatment.

Our results showed an important reduction of the PON1 activity in diabetic patients compared to the healthy group. These results were supported by Rosenblat et al. [30]. As reported in other studies [31], we showed decreased PON1 activity in diabetic patients. Besides the changes in lipid parameters, metabolic abnormalities observed in DM affect the reduction of the antioxidant capacity of HDL, and also the decreasing PON1 activity via changes in the activity of lipoprotein lipase leading to accelerate atherosclerosis process [32]. The low PON1 activity decreases ability to prevent lipidperoxide formation with consequent acceleration of the oxidative stress. Overproduction of the reactive oxygen species in diabetic patients may be due to chronic hyperglycemia, hyperinsulinemia, elevated free fatty acids (FFA) and dyslipidemia [33]. Plasma lipids

### Table 2. The cardiovascular risk factors in patients with diabetes and CAD diseases.

<table>
<thead>
<tr>
<th>Cardiovascular risk factors</th>
<th>CAD patients (n = 36)</th>
<th>Diabetic patients (n = 110)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65 ± 10</td>
<td>58.30 ± 10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31 ± 1.9 (obesity)</td>
<td>29 ± 2.2 (overweight)</td>
</tr>
<tr>
<td>Hypertension (systolic and diastolic blood pressure [mmHg])</td>
<td>Hypertensive (15 ± 0.20 systolic; 9.50 ± 1 diastolic)</td>
<td>Normal (12.25 ± 1.40 systolic; 7.40 ± 0.90 diastolic)</td>
</tr>
<tr>
<td>Physical inactivity</td>
<td>All patients (100%) declare have no physical activity</td>
<td>89.09% declare that they have no physical activity</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>4.50% smoke</td>
<td>5.45% smoke</td>
</tr>
<tr>
<td>Alcohol intake (%)</td>
<td>No one</td>
<td>No one</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>No one</td>
<td>100%</td>
</tr>
<tr>
<td>Family history (%)</td>
<td>50%</td>
<td>89%</td>
</tr>
</tbody>
</table>

BMI: body mass index.
also modify composition, function and concentration of the HDL. Elevated plasma TG-rich lipoproteins may substitute cholesterol esters (CE) in HDL by driving cholesterol ester transfer protein (CETP) with subsequent HDL depletion of CE. As a result, both the conformation and function of HDL may be altered. Glycation of HDL or directly of PON1 in HDL as occurs in diabetes may result in detachment of PON1 itself from the HDL and PON1 inactivation [34]. When HDL was incubated in very high concentrations of glucose (1 mmol/L), the esterolytic PON1 activity was preserved. In contrast, HDL incubated in normal (5 mmol/L) or elevated (up to 100 mmol/L) glucose concentrations caused a loss of the esterolytic PON1 activity [24]. The low enzyme activity is caused rather by glycation of the PON1 protein than by reduced synthesis of its molecules. PON1 is bound by HDL in lesser extent in diabetic patients as compared to healthy people and its activity is then poorly stabilized. PON1 activity was found to be decreased in CVD [35]. Several factors may take part in these changes. Firstly, oxidative stress is accelerated and thus lipid peroxidation may contribute to vascular wall impairment [36]. Secondly, glycation of proteins including enzymes may decrease their activities in diabetes [37].

Conclusion

This original study reports for the first time the decrease serum PON1 activity in patients with CAD or with diabetes mellitus living in Casablanca. In summary, the hypertriglyceridermia, the decreased c-HDL, the elevated c-LDL and the significant decrease PON1 activity, confirm the increased cardiovascular risk among CAD and diabetic patients in the studied population. The role of PON1 in CAD and in type II DM is not fully understood and this work will help to consider new perspectives of research in physiopathological mechanisms of these pathologies in Moroccan subjects and the mechanism of PON1 protective action; and also suggest new treatments. Our results indicate that PON1 could be considered a biomarker of the CVD, and may be an independent risk factor in both diabetics and CAD patients. We are now conducting a study on a molecular modelling approach of the PON1 protein to explain the structure activity relationship.

Conflicts of interest: none.

References


