Decreased paraoxonase1 activity is a cardiovascular risk factor in Moroccan adults with acute coronary syndrome

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Abstract— Paraoxonase 1 (PON1) decreased activity has been associated with susceptibility to coronary heart disease (CHD). PON1 is a high-density lipoprotein (HDL)-associated enzyme capable of inhibiting atherogenesis and/or atherosclerosis progression. Several risk factors of CHD such as age, sex, diabetes, obesity, high arterial blood pressure, hyperlipidemia, smoking and family history modulate PON1 activity. In this study, we evaluated PON1 activity in a Moroccan population of 205 patients with acute coronary syndrome (ACS) and 100 healthy controls. PON1 activity was measured by following paraoxon degradation using spectrophotometry technique. Systemic oxidative stress was evaluated by measuring protein carbonyl, malondialdehyde (MDA), and vitamin E plasma levels. Our results demonstrate that compared to healthy subjects, PON1 activity and vitamin E levels were significantly lower in coronary patients ($p<0.001$ for both paraoxonase and alpha tocopherol, $p<0.05$ for gamma tocopherol), while we observed higher oxidative stress markers in ACS patients. PON1 activity decreased considerably with accumulation of more risk factors. This study also highlights an abnormal lipid profile associated with decreased PON1 activity. The impairment of this activity may be due to oxidative stress conditions in which many factors are involved.

Index Terms— PON1, HDL, ACS, oxidative stress, protein carbonyl, MDA, vitamin E, risk facrors.

1 INTRODUCTION

ATHEROSCLEROSIS triggers a variety of vascular diseases leading to increased morbidity and premature death [1]. Acute coronary syndrome (ACS) is a common complication of atherosclerosis and a life-threatening form of coronary heart disease (CHD). It includes unstable angina, non-ST segment elevation myocardial infarction (NSTEMI), and ST segment elevation myocardial infarction (STEMI). Disruption of atherosclerotic plaque and the resulting intracoronary thrombosis are thought to account for most ACS cases [2].

Paraoxonase 1 (PON1) is a serine esterase secreted by the liver, originally identified because of its role in the detoxification of paraoxon and other organophosphates [3]. In humans, it is located on chromosome 7 and is a member of a gene cluster that includes PON2 and PON3 [4]. PON1 appears to be a significant physiological regulator of oxidative stress, modulating the release of pro-inflammatory factors involved in atherosclerosis and subsequent plaque formation. PON1 is associated with high-density lipoproteins (HDL) and prevents oxidation of low-density lipoproteins (LDL) [5]. Homocysteine thiolactone, a highly reactive metabolite of homocysteine, is another endogenous substrate for PON1; hence some of the cardio-protective effects of PON1 may be partially mediated through homocysteine thiolactone clearance [6]. There is strong linkage disequilibrium across the PON1 gene, and PON1 activity has been shown to be up-regulated by the C-allele at -108 (rs705379) [7]. However, PON1 activity is also heavily modulated by environmental factors with much individual variation being independent of genotype [8], [9].

Several studies have shown that decreased PON1 activity is a cardiovascular risk factor [10]. Shih et al., [11] elegantly demonstrated how PON1 conveys the anti-atherogenic properties of HDL [11]. While high HDL levels have been associated with cardiovascular protection, the functionality of HDL is increasingly seen as important if not more important than their level in the prevention of cardiovascular events [12]. This is even truer in patients presenting a cardiovascular disease risk factor or with established CHD in whom metabolic changes, oxidative stress, and chronic inflammation affect HDL functionality [13], [14]. Indeed, dyslipidemia, considered to be one of the most prevalent risk factors for ACS, affects 30% to 50% of ACS patients [15]. Dyslipidemia remains a residual annual risk for 9% of patients with established coronary artery disease (CAD) [16].

Diabetes increases the risk of cardiovascular disease. Studies have shown lower serum PON1 activity in type 2 diabetes and familial hypercholesterolemia patients, which are associated with accelerated atherosclerosis and CAD [17], [18]. A Turkish study reported that obese subjects have increased oxidative stress and decreased PON1 activity, which might contribute to accelerated atherosclerosis. A decrease in PON1 activity seems positively correlated with body max index (BMI) and inversely correlated with HDL levels [19]. Hypertension is one of the most important risk factors for cardiovascular disease; the PON1-108 polymorphism may be associated with mean arterial

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blood pressure (MAP) in an age-dependent manner [20].

2 Materials and Methods

2.1 Subjects

Three hundred and five subjects were enrolled in our study and were distributed into two groups based on their health status. The first group consisted of 100 healthy subjects that were recruited from patients visiting the Biomedical Centre of the Casablanca Pasteur Institute in Casablanca, Morocco, for medical check-ups. These subjects (50 men and 50 women, mean age: 54.95±0.55 years) were all healthy non-smokers and were not undergoing any treatments or taking vitamin supplements. The second group consisted of 205 patients with ACS (125 men and 80 women, mean age: 57.47±0.67 years), who were enrolled at the Cardiology Department of the University Hospital Center in Casablanca, Morocco. They met the diagnostic criteria for ACS, which was characterized using electrocardiograms (ECGs) as STEMI, NSTEMI, or unstable angina. Acute myocardial infarction was confirmed with instrumental examination, including coronary angiography and echocardiography. Patients suffering from hemorrhagic or ischemic stroke, heart failure, arthritis, hypertension, or diabetes were also included. Patients with dysthyroidism, renal failure (creatinine clearance <40 ml/min), or undergoing hormonal treatment were excluded. Arterial blood pressure, lipid profile (LDL, HDL, and total cholesterol), C-reactive protein (CRP), and glucose levels were determined. The biochemical and physical characteristics of the healthy subjects and ACS patients are listed in Table 1. All participants gave written informed consent prior to taking part in the present study.

TABLE 1
DEMOGRAPHIC AND CLINICAL DATA OF ACUTE SYNDROME CORONARY PATIENTS AND CONTROL GROUP

Parameters	Healthy subjects	Coronary patients	P values
Mean age, years	54.95±0.5551	57.47 ± 0.6699	<0.05
Effectif (male/female)	50/50	125/80	
BMI, kg/ m²	24.5±0.22	27.2±0.261	<0.001
Systolic blood pressure	120.9± 0,95	132.8 ± 1.12	<0.001
Diastolic blood pressure	71.00 ± 0.61	77.02 ± 0.77	<0.001
Glucose mmol/1	5.14 ± 0.01	8.23 ± 0.049	<0.001
Cholesterol mmol/1	3.75 ± 0.08	4.68 ± 0.08	<0.001
TG, mmol/1	1.19 ± 0.03	2.15 ± 0.07	<0.001
HDL-C, mmol/1	1.25 ± 0.02	0.98 ± 0.02	<0.001
LDL-C, mmol/1	2.85 ± 0.05	3.72 ± 0.07	<0.001
CRP mg/1	6.781 ± 0.34	10.11 ± 0.76	<0.05

*Values are mean±SEM. The unpaired student t-test was applied. Significance was calculated in comparison to healthy subjects: * p<0.05, ** p<0.01, *** p<0.001. HDL-C (HDL-cholesterol), LDL-C (LDL-cholesterol), TC (total cholesterol), CRP (C reactive protein), TG (triglycerides).*

2.2 Blood sample collection and lipid profile measurements

Blood samples were collected in dry or EDTA tubes after an overnight fast. The samples were centrifuged at 3000xg for 10 min, and aliquots of plasma were immediately stored at –80°C until analyzed. Serum total glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and C-reactive protein levels were measured using automated enzymatic assays (Kodak, Ektachem USA Systems).

2.3 PON1 activities

PON1 enzymatic activity was determined by measuring 4-nitrophenol absorption at 412 nm; 4-nitrophenol results from paraoxon (O,O-diethyl-O-Pnitrophenylphosphate; Sigma) degradation by PON1 [21]. Enzymatic activity was calculated using the 17.100 M^{-1} cm^{-1} molar extinction coefficient. One unit of paraoxonase activity was defined as 1 nmol of 4-nitrophenol formed per minute.

2.4 Systemic oxidative stress markers

Systemic oxidative stress was evaluated by measuring plasma protein carbonyl, malondialdehyde (MDA), and vitamin E (α– and γ-tocopherol) levels.

Protein carbonyl: Plasma protein carbonyl levels were assayed as described by Levine et al., [22]. Briefly, carbonyl levels were determined by dinitrophenylhydrazine derivatization and were detected in TCA-precipitable materials by measuring the absorbance at 370 nm (ε = 22.000 M^{-1} cm^{-1}) [22].

Plasma MDA: Thiobarbituric acid-reactive substances (TBARS), mainly MDA, were assayed by high-performance liquid chromatography (HPLC) as described by Agarwal and Chase, [23] using a 5 µm ODS 100 mm × 4.6 mm HP Hypersil column, a 5 µm ODS guard column, and a methanol:water (40:60, v/v) mobile phase. The fluorescence detector was set at an excitation wavelength of 515 nm and an emission wavelength of 553 nm. Plasma samples were treated with BHT antioxidant and were heat derivatized at 100°C for 1 h with thiobarbituric acid at acidic pH. They were then extracted with n-butanol, and 10 µl were injected on the column [23].

Plasma vitamin E: Plasma endogenous vitamin E was assayed as α- and γ-tocopherol. Briefly, 100 µl of thawed plasma was mixed with an equal volume of ethanol, and tocopherols were extracted in 500 µl of hexane. Plasma α-tocopherol was resolved on a Sephasil reverse-phase HPLC column (C18, 5 µm particles, 25 x 0.46 cm i.d.; Pharmacia Biotech, Piscataway, NJ, USA) using a methanol-ethanol-isopropanol (88:24:10, v/v/v) mobile phase containing 20 mM lithium perchlorate and a flow rate of 1 ml/min. α-tocopherol levels were determined using an ESA Coulochem II 50-10A electrochemical cell. Ultraviolet absorption was also monitored at 292 nm [24]. Tocopherol acetate was used as the internal standard.

2.5 Statistical analysis

The statistical analysis was performed using Graph-Pad Prism5 .Values are expressed as mean ± SEM unless otherwise indicated. Comparisons between groups were performed using an unpaired t-test. One-way ANOVA was used for multiple comparisons; correlation coefficients between all parameters studied were calculated by Person's correlation analysis. P values <0.05 were considered to be statistically significant.

3 Results

The baseline characteristics of the subjects are summarized in Table 1. There was a significant difference between ACS patients and healthy subjects with respect to BMI, blood pres-

sure, CRP inflammatory marker, and lipid profile (total cholesterol, triglyceride, HDL, and LDL levels). ACS patients had low HDL (0.98±0.02 mmol/l, p<0.001) and high LDL (3.72±0.07 mmol/l, p<0.001) and triglyceride levels (2.15±0.07 mmol/l, p<0.001) (Table 1). Approximately 42% of the ACS patients were diabetics, 30% were obese, 35% were cigarette smokers, 35% were hypertensive, and 62% had a family history of ACS.

3.1 PON1 activity and oxidative stress

Compared to healthy subjects, PON1 activity (366.3±16.12 U/ml vs 210.1±6.37 U/ml (p<0.001)) and vitamin E (gamma tocopherol: 3.55±0.406 µM vs 1.95±0.457 µM (p<0.05) and alpha tocopherol: 64.4±9.72 µM vs 15.96±5.64 µM (p<0.001)) were significantly lower in coronary patients, while we observed higher oxidative stress markers (Protein carbonyl: 3.07±0.174 nmol/mg vs 9.29±0.263 nmol/mg (p<0.001)); Malondialdehyde : 2.35±0.173 nmol/mg vs 7.11±0.304 nmol/mg (p<0.001)) in ACS patients than in healthy subjects (Table 2).

TABLE 2
OXIDATIVE STRESS MARKERS IN ACS PATIENTS AND HEALTHY SUBJECTS

Parameters	Healthy subjects	Coronary patients	P values
Protein carbonyl (nmol/mg)	3.07 ± 0.174	9.29 ± 0.263	<0.001
Malondialdehyde (µM)	2.35 ± 0.173	7.11 ± 0.304	<0.001
Gamma tocopherol (µM)	3.55 ± 0.406	1.95 ± 0.457	<0.05
Alpha tocopherol (µM)	64.4 ± 9.72	15.96 ± 5.64	<0.001
Paraoxonase activity (U/L)	366.3 ±16.12	210.1 ± 6.37	<0.001

*The unpaired student t-test was applied. Significance was calculated in comparison to healthy subjects: * p<0.05, ** p<0.01, *** p<0.001.*

3.2 PON 1 activity, clinical parameters and different cardiovascular risk factors

The correlation between PON1 activity and clinical parameters, and oxidative stress markers in ACS patient's blood are shown in Table 3. A significant inverse correlation between PON1 activity and malondialdehyde was observed (r=-0.2493; p<0.0001). Furthermore PON1 activity correlated positively with systolic blood pressure (r=0.1943; p<0.01). The PON1: HDL ratio was significantly lower in the ACS patients than in the healthy subjects. There was a strong significant correlation between PON1 activity and the PON1: HDL ratio for healthy subjects and ACS patients (Figure 1). However, the coefficient value of these correlations was significantly lower in the ACS patients than in the healthy subjects (r=0.94 vs r=0.80, respectively, p<0.001) (Figure 1).

We studied the association of cardiovascular risk factors with PON1 activity per patient. We selected six factors: diabetes, hypertension, obesity, smoking, alcohol and family history (Figure 2). Our results show that the decrease in PON1 activity is related with the combination of these six risk factors. Then we assessed the impact of age, gender, smoking habit, alcohol, diabetes and hypertension on PON1 activity as shown in figure 3. We observed that paraoxonase activity decreased significantly in diabetics (189.9±9.14 vs 224.9±8.55), alcohol drinks (173.3±16.14 vs 214.6±6.89) and elderly patients (201.6±6.67 vs 241.5±15.40); however no significant change was observed in obese (208±7.74 vs 215.3±11.06), and hypertensive patients (197.5±9.77 vs 216.2±8.25) nor in smokers (215.4±8.12 vs 200.1±10.16).

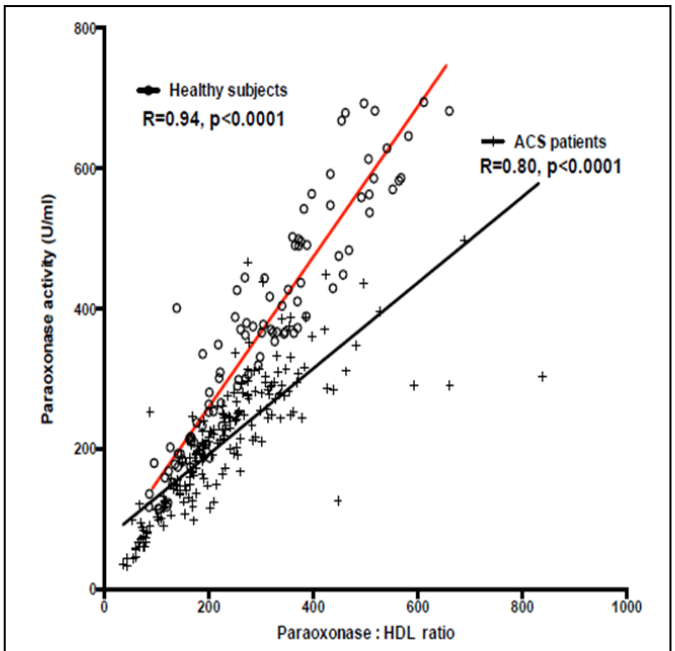

Fig. 1. Correlation between PON1 activity and PON1: HDL ratio in healthy subjects and ACS patients. Plasma paraoxonase activity was determined by spectrophotometry (R=0.94, p<0.0001 and R=0.80, p<0.0001, respectively, for healthy subjects and ACS patients).

4 DISCUSSION

Atherosclerosis and its vascular complications are a major cause of morbidity and mortality in the world. While diabetes, smoking and obesity are individual risk factors for atherosclerosis complications [25], several studies have shown that the combination of more than one risk factor accelerates atherosclerosis progression [26], [27].

In the present study, we analyzed lipid profile, oxidative stress markers and the correlation of PON1 activity and cardiovascular risk. ACS patients showed a predictable disturbed lipid profile characterized, as reported previously by hypertriglyceridemia, increased serum LDL-C levels, decreased HDL-C, which is similar to the levels reported in the literature [28], [29]. Other studies didn't show significant difference in triglycerides levels [30], [31]. Hypertriglyceridemia has been associated to a decrease of lipolytic enzymes activity, such as a lipoprotein lipase; it has been shown that the processing of TG-rich HDL by hepatic lipase can be considered as one of the mechanisms that may explain the reduction in HDL-C levels in hypertriglyceridemia [32]. Decreased HDL cholesterol levels are associated with an increased risk of coronary artery disease (CAD) in non-insulin dependent diabetes mellitus [33]. A clinical study has shown that a low HDL levels in ACS patients is a key predictor of major adverse cardiac events and death at 1 year [34]. Approximately half of ACS patients have low HDL levels, and low HDL is almost completely untreated at onset or following recurrent ACS [35]. PON1 is a protein associated exclusively with HDL. It has been suggested that

PON1 activity is as marker predicting CVD [36]. Recently a study showed that reduced PON1 activity is a good marker for severe CAD [37]. Our results confirmed the association between low PON1 activities and an increased risk of CVD. Due to the close association of PON1 with HDL particles and the importance of PON1 in the regulation of the functionality of HDL, our results suggest that the level of PON1 activity might be a strong marker of HDL antiatherogenic function and CVD risk. Decreased PON1/HDL ratio may also lead to the reduction of the antioxidant capacity of HDL [38].

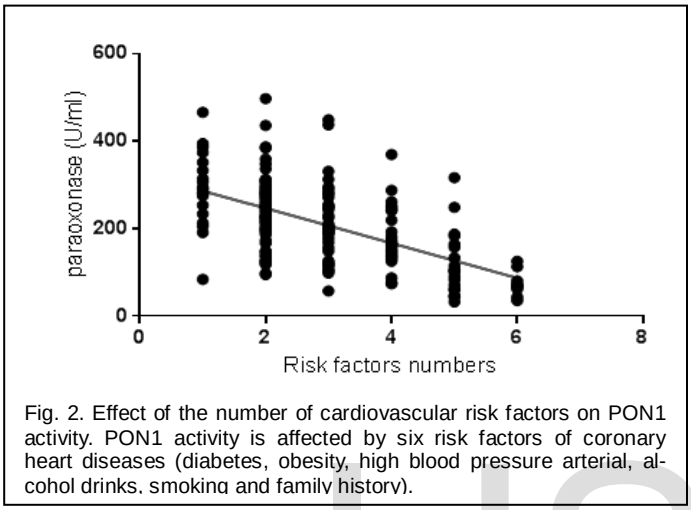

Fig. 2. Effect of the number of cardiovascular risk factors on PON1 activity. PON1 activity is affected by six risk factors of coronary heart diseases (diabetes, obesity, high blood pressure arterial, alcohol drinks, smoking and family history).

We selected three markers to evaluate oxidative stress intensity in ACS patients and the impact of oxidative stress on PON1 activity. We observed significantly higher MDA, protein carbonyl levels in the ACS patients than in the healthy subjects. Plasma vitamin E levels (α-tocopherol, γ- tocopherol) and PON1 activity were significantly lower in ACS patients than in the healthy subjects, which confirm the increase in MDA, protein carbonyl levels. A decrease in PON1 activity and vitamin E seem to be associated with increased oxidative stress [39], [40], however our results showed a significant inverse correlation between PON1 activity and malondialdehyde. The alteration of pro-oxidant/antioxidant balance, leading to inactivate PON1 activity and to the development of oxidative stress conditions which results in an increase of some oxidative stress markers like the increase in MDA, protein carbonyl levels in ACS [41], from which many factors are involved principally diabetes and smoking , a Tunisian study have shown that MDA levels were significantly higher in subjects smoking more than 40 cigarettes per day compared to those smoking less than 20 cigarettes per day. Moreover, they found that smoking multiplies by 2.8 the risk of an increase in MDA. In addition, among smokers, the risk of an increase in MDA increases with the number of cigarettes smoked per day and with consumption duration [42] although no significant correlation could be established between smokers and nonsmokers in terms of mean total antioxidant status (TAS), total oxidant status (TOS) and PON1, it is a fact that TAS, TOS and PON1 in the organism are affected by many factors like hypertensive and diabetes and therefore there is a need for more extensive studies in this regard [43]. Our results showed that PON1 activity was significantly lower in ACS patients with diabetes type 2, in patients older than fifty years, and in patients who

TABLE 3
CORRELATION COEFFICIENTS BETWEEN LOG (PON1) ACTIVITY AND DIFFERENT PARAMETERS

Parameters	r	P values
Age (y)	-0.0586	0.2018
BMI, kg/m²	0.0925	0.1871
Systolic blood pressure	0.1943	0.0052*
Diastolic blood pressure	0.08290	0.2373
Cholesterol (mmol/l)	0.05580	0.4268
TG (mmol/l)	-0.02552	0.7165
HDL-C (mmol/l)	0.05843	0.4053
LDL-C (mmol/l)	-0.08573	0.2217
Protein carbonyl (nmol/mg)	0.06043	0.3894
Malondialdehyde (µM)	-0.2493	0.0003*
Gamma tocopherol (µM)	0.1744	0.1581
Alpha tocopherol (µM)	0.2281	0.0633
CRP (mg/l)	-0.1049	0.3041

significant correlation with spearman's correlation coefficient.

drink alcohol. Several studies have shown the reduction of PON1 activity in diabetic patients [44], [45]. The atheroprotective properties of HDL are also affected under oxidative stress conditions in diabetic patients [46], [47]; little information is available on the mechanism that may explain this, and thus, the decrease in PON1 activity may contribute to increased susceptibility of HDL to oxidation with aging. Altogether, this suggests that the decrease in PON1 activity may be related to the development of oxidative stress conditions with aging and the increased HDL susceptibility to oxidation and may contributes to the acceleration of the atherosclerosis process in elderly subjects [48], [49]. The dramatic increase of CVD with age could be due to the increased susceptibility of LDL and HDL to oxidation as shown in our previous studies [50], [51]. However, a decrease in the specific antiatherogenic activity of HDL with aging might also contribute to increase CVD. PON1 has been shown to be mostly responsible for the antioxidant activity of HDL [52]. Thus, a reduction in the antioxidant potential of PON1 might also influence the susceptibility of LDL and HDL to peroxidation as well as the antioxidant properties of HDL.

Obesity is associated with major risk factors for atherosclerosis including hyperlipidemia, diabetes, hypertension, and metabolic syndrome [53]. Obesity and oxidative stress have been shown to play an essential role in the pathogenesis of atherosclerosis. Moreover, oxidative stress has been reported to be involved in the pathogenesis of various diseases such as hyperlipidemia, diabetes, hypertension, which are also associated with obesity and atherosclerosis [54]. In our study there was no significant difference in PON1 activity between obese and non-obese patients. Several experimental and clinical trials have shown that serum PON1 activity is decreased in obese subjects [55], [56]. However, we still have limited knowledge about the association between serum PON1 activity and obesity [56], [57]. Studies evaluating the association

between serum PON1 activity and obesity were conducted in children [58], [59], [60].

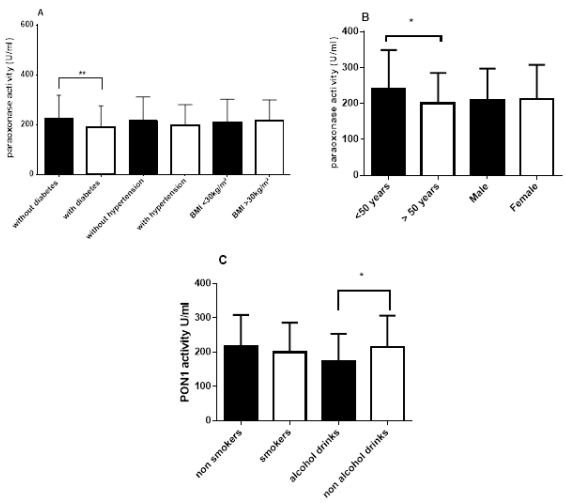

Fig. 3. Variation of PON1 activity with cardiovascular risk factors in ACS patients A) Variation of PON1 activity in ACS patients with diabetes, hypertension, and obesity factors.B) Variation of PON1 activity in ACS patients with age, and gender factors.C) Variation of PON1 activity in ACS patients with alcohol drink, and smoking factors.

Light to moderate alcohol consumption has been widely established to be protective against coronary heart disease (CHD), whereas heavy alcohol consumption has been shown to have a potential detrimental effect [61]. The reduction in risk of CHD associated with light and moderate alcohol intake is generally attributed to the beneficial effects of alcohol on high-density lipoprotein (HDL) cholesterol levels. Alcohol consumption has been shown to affect lipoprotein metabolism, hemostasis, and vascular wall functioning, with the protective effects of light and moderate alcohol consumption partially explained by increased plasma high-density lipoprotein (HDL) cholesterol [62], [63]. Decreased PON1 activity is suggested to be associated with an increased risk of cardiovascular disease [64]. Studies in both humans and rats have demonstrated low amounts of alcohol to increase PON1 levels and high amounts of alcohol to decrease PON1 levels compared with no alcohol intake [65], [66].

4 Conclusion

This comparative study of 205 ACS patients and 100 volunteers found an abnormal lipid profile associated with a decrease in PON1 activity. The impairment of this activity of PON1 may be due to oxidative stress conditions for which many factors are involved like diabetes, smoking, alcohol consumption and obesity.

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