

Paraoxonase 2 Gene (Cys³¹¹-Ser) **Polymorphism and the Risk of Coronary Artery Disease**

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Abstract

Background: Human paraoxonase-2(PON2) which is exclusively intracellular possesses unique properities that distinguish it from PON1 and PON3. Recently, it was demonstrated that PON2 protects against atherosclerosis by preventing LDL oxidation. Emerging evidences have proposed that genetic variations in the PON2 gene may be associated with coronary artery disease (CAD). Objectives: To investigate the relationship between a common PON2 gene (Cys³¹¹-Ser) polymorphism and the presence and extent of CAD. Methods: The study comprised 112 patients recruited from those undergoing coronary angiography for suspected CAD, who were divided according to the presence or absence of CAD into 2 groups Group I including 62 patients with CAD and Group II including 50 patients proved to have normal coronaries. All the subjects included in the study were genotyped for the (Cys³¹¹-Ser) polymorphism of PON2 gene using RCR-RFLP. Results: The frequency of Cys allele was significantly higher in group I compared to Group II (77.4% vs. 56% respectively, P < 0.01). Patients with vessel score 3 had significantly higher severity score and higher Cys allele frequency than patients with vessel score 2, the latter group had also significantly higher severity score and Cys allele frequency than patients with vessel score 1. In multivariate logistic regression analysis of different variables for prediction of CAD, age [OR 3.79, CI (1.33 - 12.7), P < 0.01], smoking [OR 0.71, CI (0.23 - 7.81), P < 0.001], and PON2³¹¹ Cys allele [OR 5.67, CI (1.99 -14.77), P < 0.001] were significantly independent predictors of CAD. Conclusion: Cys allele of PON2 311 gene polymorphism is an independent risk factor for CAD and it is associated not only with the presence of CAD but also with its extent and severity.

Keywords

Paraoxonase 2, Coronary Artery Disease, Polymorphism, Atherosclerosis

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1. Introduction

Coronary Artery Disease (CAD) continues to be the leading cause of morbidity and mortality in developed countries [1]. Atherosclerosis, its pathogenic mechanism, is a progressive disease characterized by entrapment of LDL particles in the vessel wall with subsequent oxidative modification, and stimulation of proinflammatory gene expression leading to inflammatory cell recruitment, infiltration and activation [2].

One of the main antioxidant enzyme systems for LDL particles is paraoxonase family (PON1, PON2 and PON3) PON1 and PON3 form part of HDL particles, whereas PON2 is found in a large variety of tissues, such as endothelial cells, smooth muscle cells and macrophages where it acts as intracellular antioxidant [1].

Although PON2 has a similar structure to that of PON1 based on high amino acid sequence homology, PON2 possesses biological functions that are distinct from PON1. PON2 cannot hydrolyze organophosphates, such as paraoxon, but instead, possesses hydrolase and lactonase activities [3].

Both *in vivo* and *vitro* models have revealed that PON2 prevents LDL oxidation, reverses the oxidation of mildly oxidized LDL, inhibits oxidized LDL inducing monocyte chemotaxis, increases cholesterol efflux and decreases the size of atherosclerotic lesions [4]. Furthermore, the antiapoptotic capability of PON2 has been found to play a role in atherosclerotic protection [5].

The human PON2 gene is located on the long arm of chromosome 7q 21.3 and is adjacent to PON gene family members PON1and PON3 [6]. Several polymorphisms in the PON2 gene have been reported to date; however, only two common PON2 polymorphisms play a prominent role in pathophysiological conditions. Codon 148 is either an alanine or a glycine (A148G), and codon 311 is either a cysteine or a serine (C311S) [3]. Many studies reported an association between Cys311Ser polymorphism of PON2 and the risk for CAD [7]. It was suggested that this polymorphism has a relation with enhanced PON2 enzyme activity which reduces LDL oxidation and increases microphage PON2 expression with a selected antioxidant response at the cellular level and exerting an anti atherogenic role by attenuating microphage foam formation [1]. The present study aimed to investigate the relationship between Cys311Ser polymorphism and the development and extent of CAD.

2. Subjects and Methods

The study comprised 112 patients recruited from those undergoing coronary angiography for suspected CAD, who were divided according to the presence or absence of CAD in to 2 groups: Group I included 62 patients with CAD and Group II included 50 with angiographically normal coronaries. All the patients in this study had given informed written consent before blood sampling. Approval was obtained from the research ethics committee.

Inclusion criteria: patients included in the study were those undoing diagnostic coronary angiography for suspected ischemic heart disease with one or more of the following

- Typical chest pain
- ECG changes
- Previous admission to hospital by CAD
- Previous cardiac intervention (PCI), coronary artery bypass graft (CABG)
- Other investigations suggesting CAD such as positive exercise test, positive myocardial perfusion scanning or echocardiographic regional wall motion abnormality suggesting CAD

Exclusion criteria:

- Diabetes mellitus
- Patients with Renal & hepatic impairment
- Presence of inflammatory and autoimmune diseases

Patients were included irrespective of traditional risk factors of CAD such as cigarette smoking, hypertension, over weight/obesity and hyperlipidemia. The presence of traditional risk factors was characterized on the basis of the European Atherosclerosis Society standards and recommendations [8].

Hypertension was defined as a systolic blood pressure of more than 140 or diastolic blood pressure of more than 90 mmHg in at least two separate measurements, diagnosis of hypotension was accepted if an individual was on pharmacological treatment and the review of medical records indicated justification for treatment.

Overweight was defined as a body mass index (BMI) greater than 25 Kg/m² and obesity as BMI greater than 30 kg/m^2 .

Hypercholesterolemia was considered to be present if total serum cholesterol levels were $\geq 200 \text{ mg/dL}$ or if the patient was undergoing treatment with cholesterol-lowering drugs [9].

Current cigarette smoking was defined as a daily intake of more than five cigarettes for more than one year [10].

All the patients in this study were subjected to:

1) Complete history taking

2) Thorough clinical evaluation

3) 12-lead resting ECG

4) Conventional echocardiographic examnation: Echocardiography was performed with the patient in the left lateral decubitus position. The equipment used was Vivid S 5 system. Measurements were performed according to the recommendations of the American Society of Echocardiography [11]. With the use of the long axis parasternal, apical 4, 5 and 2-chamber views. All patients underwent conventional M-mode and 2-D echocardiographic examination. Visual assessment of regional wall motion was performed. Endsystolic volume, end diastolic volume (by manual tracing of endocardial borders) and ejection fraction of each ventricle was calculated using Simpson's rule [12]. Convensional (continuous-wave and color) Doppler valvular flow was determined.

5) Angiographic analysis: Diagnostic coronary angiography (CA) was carried out in all patients using Judkins technique. Quantitative analysis of coronary arteries was performed with the computer-assisted coronary angiography analysis system. End-diastolic frames from each arteriogram were selected for analysis [13]. The percentage diameter stenosis (DS) was assessed in different projections and the highest value of each lesion was chosen. Images of the coronary tree were obtained with the digital Philips set system and reviewed by an experienced cardiologist who had no knowledge of the patients' biochemical results to assess the extent and severity of CAD and morphology of all coronary artery stenoses.

The coronary tree was divided into 16 segments as follows [14]:

- The left main coronary artery (LMCA) as one segment
- The left anterior descending (LAD) artery was divided into: proximal, mid, distal segments beside two diagonals DI and D2
- The left circumflex artery (LCX) artery was divided into: proximal, mid, distal segments beside two marginal branches OMI and OM2
- The right coronary artery (RCA) artery was divided into: proximal, mid, distal segments beside posterior descending artery (PDA) and postrolateral (PL) branch

Coronary angiograms were scored according to:

1) Vessel score [15]: This was the number of vessels with a significant stenosis (50% or greater reduction in lumen diameter). Degree of stenosis was defined as the greatest percentage reduction of luminal diameter in any view compared with the nearest normal segment and was determined visually. Scores ranged from 0 to 3, depending on the vessels involved. Left main artery stenosis was scored as single vessel disease [15].

2) Severity score [16]: the coronary circulation was divided into eight proximal segments. Disease in the distal segments was not considered because of difficulty in quantitating the severity of lesions in these areas. The eight proximal segments scored included the left main coronary artery, the left anterior descending artery (LAD) up to the junction of the middle and distal third of the vessel, the proximal third of the major septal branch of the LAD, the proximal third of the major diagonal branch of the LAD, the left circumflex coronary artery (LCX) up to the junction of the middle and distal thirds of the vessel, the proximal third of the major obtuse marginal branch of the LCX, the right coronary artery (RCA) up to and including the origin of the posterior descending coronary artery (PDA), and the proximal third of the PDA. In cases in which the PDA was supplied by the LCX vessel (LCX dominance), lesions in the LCX up to the origin of the PDA were included, as were lesions of the RCA up to the origin of the middle and distal thirds of the vessel. The PDA was scored identically for RCA and LCX dominant circulations. The percentage by which each lesion in the proximal coronary circulation narrowed the artery was assessed according to the maximal narrowing of the diameter of the artery in all projections. The severity of the proximal coronary disease was assessed by assigning points to each lesion as follows: less than 50% stenosis of the luminal diameter, 1 point; 50% to 74% stenosis, 2 points; 75% to 99% stenosis, 3 points; total obstruction, 4 points. The points for each lesion in the proximal coronary circulation were summed and a score for severity of coronary atherosclerosis was obtained. In previous study, the coefficient of variation between two angiograms analyzed several months apart without knowledge of the previous score was 4.9% [16].

Lipid profile including serum cholesterol [17], triglycerides [18], HDL-c [19] and LDL-c [20] (by enzymatic colorimetric test), genotyping for Cys311Ser polymorphism of the PON2 gene were done.

Blood sampling: Ten ml of fasting (12 - 14 hour) venous blood were withdrawn from the cubital vein of every patient. Five ml were transferred slowly into vacunated EDTA tube for isolation of white blood cells for genotyping. Five ml were transferred slowly into a plain tube for determination of serum total cholesterol, trigly-cerides and HDL cholesterol and left for 30 min for clotting and centrifuged for 10 min at 4000 r.p.m. The serum obtained frozen at -20° C till analysis. Serum cholesterol, triglycerides and HDL cholesterol were determined by enzymatic colorimetric test. LDL cholesterol was estimated by Friedewald's formula.

Leukocyte isolation: Leukocytes were isolated from the whole blood by removing erythrocytes by suspending cells in 8 ml erythrocyte lysing buffer (ELB), then centrifugation for 5 min at 1000 rpm. Centrifugation was repeated twice more till a white pellet appeared. Carefully, the blood platelets were removed and the white pellet was washed twice with 1 ml phosphate buffer solution (PBS) and then was transferred to eppendorf tubes for next step DNA extraction [21].

DNA Extraction: By Using QIAamp[®] DNA Blood Mini Kits cat# 51104 (QIAGEN HILDEN, Germany). Genotyping for Cys³¹¹-Ser polymorphism of the PON2 gene:

The amplification reaction was done in 25 μ l final volume (10 μ l DNA template + 15 μ l master mix containing 2.5 μ l 10× PCR buffer, 1.5 μ l MgCl 25 mM, 0.5 μ l dNTPs 10 mM, 0.5 μ l Taq polymerase 5 U/ μ l, 1.0 μ l forward primer 50 mM {F5'-ACATGCATGTACGGTGGTCTTATA-3'}, 1.0 μ l reverse primer 50 mM {R5'-AGC-AATTCATAGATTAATTGTTA-3'} and 8 μ l distilled water.

PCR amplification was done using Perkin Elmer thermal cycler 2400 (USA). PCR conditions were as follow: After initial denaturation (95°C for 5 min); 40 cycles were run at the following conditions: 95°C denaturation for 1 min, 50°C annealing for 1.5 min, 72°C extension for 1.5 min; a final cycle of 72°C for 7 min.

Recognition Site for DdeI:

5'.... C V TNAG.... 3'

3'... GANT▲C... 5'

The PCR product (262 bp) was digested overnight with 2U DdeI restriction enzyme (BioLabs, New England, cat# R0175S) at 37°C. The DdeI digestive products were run by 4% agarose gel electrophoresis for 30 min and stained with ethidium bromide. The bands were visualized under ultraviolet light. Fragment size of 142 bp and 120 bp corresponds to Cys 311 (C allele), whereas 75 bp and 67 bp are diagnostic bands for ser 311 (S allele). Note that a 120 bp band is present in all genotypes because of the presence of a common Dde1 site [2].



Restriction patterns of the PCR-amplified 262-bp fragment after digestion with DdeI. Lane 1: 50 bp ladder. Lane 2, 6, 7, 10: CC genotype. Lane 3, 9: SS genotype. Lane 4, 5, 10, 9: CS genotype.

Statistical analysis:

Statistical analysis was made by IBM computer with the aid of Microsoft Excel and some statistical software programs like statistical program of social sciences version 11.5 (SPSS V. 11.5), Epicalc 2000, Epiinfo 2000.

Qualitative data were expressed in the form of number and percentage. Quantitative data were expressed as mean and standard deviation. The following statistical tests were used: Chi-square test is a two-way and multi-way test of significance that was used to figure out association between two or more qualitative variables. The t-test assesses whether the means of two groups are statistically different from each other. This analysis is ap-

propriate whenever you want to compare the means of two groups. Anova (F) test: the One-Way ANOVA procedure produces a one-way analysis of variance for a quantitative dependent variable by a single factor (independent) variable. Multivariate logistic regression analysis is used to assess the association of PON2 polymorphism and development of CAD.

3. Results

The study include 112 patients recruited from those undergoing coronary angiography, who were divided according to the presence or absence of CAD into CAD^{+ve} Group (I) (n = 62, they were 42 males and 20 females with mean age 54.95 ± 5.42 years) and CAD^{-ve} Group (II) (n = 50, they were 23 males and 27 females with mean age 52.98 ± 5.62 years).

Concerning patient characteristics, there was no significant statistical difference between CAD^{+ve} and CAD^{-ve} groups regarding age (P > 0.05) while there was significant statistical difference between the two groups regarding sex with predominance of male sex in CAD^{+ve} group (P < 0.05) there was 2.47 fold increased risk for CAD in male sex (OR: 2:47 95% CI 1.14 - 5.32) (Table 1).

Concerning the distribution of risk factors among the two groups, BMI was significantly higher in CAD^{+ve} group compared to CAD^{-ve} group (P < 0.05). Also, the number of smokers was significantly higher in CAD^{+ve} group (P < 0.05) while there was no significant statistical difference between the two groups regarding the presence of hypertension (P > 0.05) (Table 1).

The biochemical laboratory profile showed that serum total cholesterol, triglycerides and LDL cholesterol levels were significantly higher in CAD^{+ve} group compared to CAD^{-ve} group while serum HDL cholesterol levels were significantly higher in CAD^{-ve} group (P < 0.001) (Table 2).

For the Cys³¹¹-Ser polymorphism of PON2 gene, 14 patients (22.6%) of CAD^{+ve} group were SS genotype, 30 patients (48.4%) were CS and 18 patients (29%) were of CC genotype. There was significant difference between the two groups regarding genotype distribution with predominance of CC genotype in CAD^{+ve} group (P < 0.001). There is 6.41 fold increased risk for development of CAD in CC genotype subjects (OR: 6.41 95% CI: 1.76-23-3).

The frequency of PON2 C-allele was significantly higher in CAD^{+ve} than CAD^{-ve} group (P = 0.01) (**Table 3**). The CAD^{+ve} group was further subclassified according to the genetic variants into 3 groups: SS, CS and CC groups. The SS group included 14 patients with mean age 53.64 \pm 5.54. They were 9 male (64.2%) and 5 females (35.8%). The CS group included 30 patients with mean age 55.27 \pm 4.7. They were 21 males (70%) and 9

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Parameters —	CAD ^{+ve} gr	oup (n = 62)	CAD ^{-ve} gro	oup (n = 50)	- Test of significance	P-value
Age (years) (SD)	Age (years) (⁻ SD) 54.95		52.98	± 5.62	*t = 1.88	0.06
BMI (⁻ SD)	28.87 ± 3.73		27.57	± 2.89	*t = 2.03	0.04
	No.	%	No.	%		
Sex:					$**\gamma^2 = 5.37$	
Male	42	67.7	23	46.0	OR (95% CI)	0.02
Female	20	32.3	27	54.0	= 2.47 (1.47 - 5.32)	
Hypertension:					$**v^2 - 2.12$	
Present	20	32.3	10	20.0	$\chi = 2.12$ OR (95% CI)	0.02
Absent	42	67.7	40	80.0	= 1.90 (0.79 - 4.56)	
Smoking:					$**2^{2} - 3.07$	
Present	26	41.9	12	24.0	χ = 3.97 OR (95% CI)	0.02
Absent	36	58.1	38	76.0	= 2.29 (0.01 - 3.8)	

*t = student t-test; ** χ^2 = chi-squared test.

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Parameters	Studied patie	- Student t-test	P-value	
T urumotors	CAD^{+ve} group (n = 62) (\overline{SD})	CAD^{-ve} group (n = 50) (^{-}SD)	Student t test	1 value
Serum cholesterol (mg/dl)	253.71 ± 48.69	183.2 ± 15.67	9.80	< 0.001
Serum triglycerides (mg/dl)	147.11 ± 28.73	101.52 ± 22.49	9.18	< 0.001
Serum HDL (mg/dl)	32.19 ± 1.46	42.56 ± 3.19	22.83	< 0.001
Serum LDL (mg/dl)	195.9 ± 43.92	123.2 ± 12.08	11.30	< 0.001

Table 2. Differences in the biochemical laboratory profile between CAD^{+ve} and CAD^{-ve} groups.

Table 5. Association of FON2 genotype and coronary aftery disease	Ta	abl	le 3	. /	Asso	ocia	atio	ı of	'P(ЭN	12	genot	ype	and	coronary	artery	disease
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		Studied subje	ects (n = 112)					
Parameters	CAD ^{+ve} gro	oup (n = 62)	CAD^{-ve} group (n = 50)		$^{*}\chi^{2}$	OR (95% CI)	P-value	
	No.	%	No.	%				
PON2 genotype:								
SS	14	22.6	22	44.0	5.82	0.37 (0.16 - 0.84)	0.01	
CS	30	48.4	25	50.0	0.03	0.94 (0.44 - 1.98)	0.87	
CC	18	29.0	3	6.0	9.64	6.41 (1.76 - 23.3)	< 0.001	
C-allele frequency	48	77.4	28	56	5.82	0.37 (0.16 - 0.84)	0.01	

females (30%) and the CC group included 18 patients with mean age 55.44 \pm 6.17. They were 12 males (66.6%) and 6 females (33% - 3%). There was no significant difference between the three genotype groups regarding age, male sex, BMI, smoking habit and lipid profile including total cholesterol, triglycerides, HDL and LDL levels (P > 0.05) (Table 4).

Regarding the number of vessel affected, there was significant statistical difference between the three genotypic groups with predominance of 3 vessel affection in the CC group (P < 0.05) (Table 5).

Multivariate logistic regression analysis for the relationship between the Cys311Ser polymorphism of PON2 gene and the development of CAD and showed that after adjustment for other risk factors such as age, male sex, BMI and smoking showed that PON2 polymorphism still have independent effect on the development of CAD (Table 6).

4. Discussion

Endothelial dysfunction is considered an early stage in the atherogenic process, however, the precise pathogenesis of transition from normal to abnormal endothelial function remains unknown [22].

Although traditional risk factors, such as hypertension and hyperlipidemia, are associated with endothelial dysfunction, the magnitude of endothelial dysfunction in patients with similar risk factors is highly variable [23]. Novel risk factors, such as oxidative stress and genetic heterogeneity, could contribute to this process, possibly mediating between traditional CAD risk factors and endothelial dysfunction [24].

Oxidative stress, which represents an imbalance between oxidant and antioxidant processes, can occur systemically or locally in the vessel wall [10]. One of the most important systems implicated in this process is the paraoxonase system [25]. The paraoxonase (PON) gen family consists of three conserved genes (PON1, PON2 and PON3) that are located on chromosome 7q21.3 - 22.1 and share a high degree of identity [26].

Studies in the last five years have focused on PON2 because of its unique expression and localization. In contrast to PON1 and PON3 which are exclusively found in the liver and are associated with HDL in the blood stream following secretion [3]. PON2 is an intracellular protein that is widely expressed in many tissues including the liver, kidney, lung, heart, placenta, testis, stomach, spleen, pancreas, small intestine, skeletal muscle, artery wall cell and macrophages [27]. Several polymorphisms in the PON2 gene have been reported, however,

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Falanciers -	SS (n = 14) (⁻ SD)		CS (n = 30) (⁻ SD)		CC (n =	CC (n = 18) (SD)		I -value	
Age (years)	53.64	± 5.54	55.27	' ± 4.97	55.44 ± 6.17		0.52	0.59	
BMI (kg/m ²)	28.1 ± 3.07		28.78 ± 3.89		29.63 ± 3.99		0.67	0.52	
Serum cholesterol (mg/dl)	251.43 ± 39.59		251.83 ± 40.55		258.61 ± 66.84		0.13	0.88	
Serum triglycerides (mg/dl)	136.36 ± 25.38		150.47 ± 30.75		149.89 ± 27.06		1.28	0.29	
Serum HDL (mg/dl)	32.07 ± 1.73		32.33 ± 1.45		32.06 ± 1.3		0.26	0.77	
Serum LDL (mg/dl)	192.07 ± 37.78		192.63 ± 41.02		203.22 ± 53.59		0.38	0.69	
	No.	%	No.	%	No.	%			
Sex:									
Male	13	92.9	18	60	8	44.4	0.10*	0.01	
Female	1	7.1	12	40	10	55.6	8.12	0.01	
Smoking:									
+ve	8	57.1%	8	26.7%	10	55.6%	*	0.06	
-ve	6	42.9%	22	73.3%	8	44.4%	5.57	0.06	

Table 4. Differences in risk factors among the different PON2 genotype.

 $\sqrt[*]{\chi^2}$ = chi-squared test.

Table 5. Paraoxonase 2 allele frequencies in different vessel scores.

Stenosis	SS (n	= 14)	CS (n	CS (n = 30)		CC (n = 18)		P-value
	No.	%	No.	%	No.	%		
One vessel	7	50	6	20.0	2	11.1	1.34	0.04
Two vessels	5	35.7	16	53.3	5	27.8	4.74	0.09
Three vessels	2	14.3	8	26.7	11	61.1	6.97	0.001

Table 6. Multivariate regression analysis of predictors of coronary artery disease in studied populations.

Itomo	Presence of CAD						
items	OR	95% CI	P value				
Age (years)	3.79	(1.33 - 12.7)	P < 0.01				
Sex (male/female)	1.27	(0.52 - 3.9)	NS				
Smoking	0.71	(0.23 - 7.81)	P < 0.001				
BMI	1.77	(1.15 - 4.99)	NS				
Hypertension	2.79	(1.95 - 4.11)	NS				
LDL Cholesterol > 100 mg/dL	4.31	(1.25 - 12.5)	P < 0.001				
HDL Cholesterol < 40 mg/dL	5.11	(1.79 - 16.33)	P < 0.001				
Paraoxonase 311 C allele	5.67	(1.99 - 14.77)	P < 0.001				

OR, odds ratio; CI, confidence interval; BMI, body mass index; HbA_{1c}, glycated hemoglobin; NS, non significant; LDL, low density lipoprotein; HDL, high density lipoprotein.

only two common polymorphisms play a prominent role in pathophysiological conditions. Codon 148 is either an alanine or a glycine (A148 G) and codon 311 is either a cysteine or a serine (Cys³¹¹Ser) [3].

The aim of this study was to investigate the relationship between the Cys³¹¹Ser polymorphism of the PON2 gene and the development of CAD and whether it has relation to the extent of the condition.

Concerning the Cys³¹¹Ser polymorphism of PON2 gene, there was significant statistical difference between CAD^{+ve} and CAD^{-ve} groups between the regarding genotype distribution with predominance of CC genotype in CAD^{+ve} group. There was 6.41 fold increased risk for development of CAD in CC genotype (OR: 6.41 94% CI: 1.76 - 23.3). Also, the frequency of C allele was significantly higher in CAD^{+ve} group.

This was is agreement with Wang *et al.* [28], Marlenlli *et al.* [29] and Jililian *et al.*, [2] who stated that CAD^{+ve} patients had a significantly higher frequency of the CC genotype compared to controls and that PON2 C-allele was significantly higher in cases than controls. But this was in contrast to Sanghera *et al.* [30], Leus *et al.* [31] and Pan *et al.* [32] who demonstrated that PON2 Ser³¹¹ carriers were at risk of CAD and that subjects with homozygous Cys genotype were probably protected against the development of CAD.

However, Robertson *et al.* [33] and Wheeler *et al.* [34] failed to show any association between $Cys^{311}Ser$ polymorphism of PON2 gene and the risk for CAD development. Souza *et al.* [35] also found no relationship between PON2 C³¹¹S SNP and neither the development of CAD nor the atherosclerotic biomarkers in the patients.

The CAD^{+ve} group was subclassified according to their genetic variants into: SS, CS and CC group. There was no significant difference between the three genotypes regarding the distribution of traditional risk factors including age, male sex, BMI, Smoking habit and all parameters of lipid profile including serum cholesterol, triglycerides, LDL and HDL levels. Martinelli *et al.* [29] stated that gender, smoking habits and other CAD-risk related variables are suggested to interact with PON2 variants to increase the risk for CAD. This was in contrast to Pan *et al.* [32], Chi *et al.* [36] and Souza *et al.* [35] who didn't find any relationship between these variables and PON2 polymorphism.

In view of the importance of PON2 in lipid metabolism, the relationship between Cys³¹¹Ser polymorphism of the PON2 and plasma lipoproteins has been examined by number of studies. Chen *et al.* [7] observed that carriers of Ser³¹¹ and Cys³¹¹Ser had significantly higher plasma total and LDL-cholesterol than subjects carrying the Cys³¹¹ genotype. While Leus *et al.* [31] reported that PON2 311 Cys had the highest plasma concentration of total cholesterol and LDL-C. Similar result were obtained by Shin [37] who found significant difference between the genotype groups regarding lipid profile with higher concentration of LDL-c in patients homozygous for ³¹¹C allele.

However, others failed to find any statistically significant association between Cys³¹¹Ser polymorphism and serum lipids and lipoprotein levels [38]. However, none of these studies examined the level of the oxidized lipids.

These discrepancies suggest that polymorphism in the PON gene are association with significant variations in intermediate traits in plasma lipoprotein metabolism, through an unknown mechanism [37].

Regarding the degree of vessel affection, results of this study showed significant statistical difference between the genetic variant groups regarding the number of vessel affected with prevalence of 3 vessel affection in the CC group.

This was in agreement with Jillian *et al.* [2] who found that the genotype frequencies were significantly different between one, two and three-vessel diseased groups with significant association with an increasing number of diseased vessel for the PON Cys³¹¹ allele. In contrast to Bayrak *et al.* [39] who found no association between PON2 Ser³¹¹Cys polymorphism and the extent and severity of CAD.

Studies in animals have also provided the information that PON2 has been shown to provide protection against HDL and LDL oxidation and inhibit monocyte transmigration in response to LDL oxidation in PON2-knockout mice [4]. Interestingly, these PON2 knockout mice demonstrate an increased number of foam cells and lipid droplets and develop significantly larger atherosclerotic lesions in comparison to wild-type counterparts [40].

In an attempt to explain the relationship between PON2 gene polymorphism and risk for CAD, Sanghera *et al.* [30] has suggested that PON2 allele merely act as a marker for an unknown functional variant and in linkage disequilibrium with other functional mutations on one of the PON family genes. Pan *et al.* [32] had linked the functional aspect of Cys-Ser substitution in lipid peroxidation to the tendency towards the development of atherosclerotic plaques.

Imai et al. [41] have suggested that this polymorphism may affect the paraoxonase concentration and enzyme

activity. Although several studies had linked the effect of genetic variants of Q/R 192 polymorphism to altered PON1 enzyme activity, a major limitation about PON2 is the difficulty to assess the enzyme concentration and activity [42]. This may be because the true biological substrate of PON2 remains unknown. Currently, the activity of PON2 is determined by the hydrolysis of dihydro coumarin (DHC), and the appropriateness of this substrate is still subjected to debate [3]. Shiner *et al.* [43] have shown that PON2 expression increases in monocytes during their maturation into macrophages as a result of NADPH oxidase activation and this process is partially regulated by the transcription factor AP-1. The stimulation of PON2 may represent a compensatory mechanism against the superoxide dismutase radicals accumulation in cells and thereby protection against atherosclerosis.

5. Study Limitations

Although the number of patients analyzed was not large in our study, the highly significant results suggest an important role of the PON2 gene Cys³¹¹Ser polymorphism in the pathogenesis of CAD but large scale studies are strongly recommended for more validation of the results. Another limitation we did not measure was paraoxonase activity. However, doing so might not clarify the complex causal relationship between paraoxonase activity and early atherosclerosis. Furthermore, paraoxonase levels in serum might not correlate with lipoprote-in-bound paraoxonase. Genetic associations are more likely to clarify the causal relationship.

6. Conclusion

Cys allele of PON2 311 gene polymorphism is an independent risk factor for CAD and it is associated not only with the presence of CAD but also with its extent and severity.

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