# ASSOCIATION BETWEEN THE SEVERITY OF ANGIOGRAPHIC CORONARY ARTERY DISEASE AND PARAOXONASE-1 PROMOTER GENE POLYMORPHISM T(-107)C IN IRANIAN POPULATION

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**Abstract-** The oxidation of low-density lipoproteins and cell membrane lipids is believed to play an integral role in the development of fatty streak lesions, an initial step in coronary artery disease (CAD). Paraoxonase-1 (PON1) is an enzyme associated with the high-density lipoprotein (HDL) particle. PON1 protects LDL from oxidative modification by hydrolyzing lipid peroxides, suggestive of a role for PON1 in the development of CAD. The present study tested the hypothesis that Paraoxonase-1 promoter polymorphism T(–107)C could be a risk factor for severity of CAD in Iranian population. Paraoxonase-1 promoter genotypes were determined in 300 consecutive subjects (> 40 years old) who underwent coronary angiography (150 subjects with >50% stenosis served as cases [$CAD^+$] and 150 subjects with < 20% stenosis served as controls [$CAD^-$]). PON1 promoter genotypes were determined by PCR and BSTU1 restriction enzyme digestion. $CAD^+$ Subjects did not show any significant differences in the distribution of PON1 promoter genotypes as compared to $CAD^-$ Subjects ($P = 0.075$). However the analysis of PON1 promoter genotypes distribution showed a higher percentage of (-107) TT among $CAD^+$ compared with $CAD^-$ ($P = 0.027$). After controlling for other risk factors, the T(–107)C polymorphism had interaction with age ($P = 0.012$), but did not show any interaction with other risk factors such as BMI ,gender, smoking, diabetes, level of HDL-C, LDL-C, triglyceride and Total cholesterol. These data suggest that the TT genotype may represent a genetic risk factor for Coronary artery disease in Iranian population.



*Acta Medica Iranica*, 46(3): 197-202; 2008

**Key words:** Promoter gene polymorphism, paraoxonase-1, coronary artery disease

Received: , Revised: , Accepted:

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## INTRODUCTION

Coronary artery disease (CAD) is a major cause of mortality and morbidity in developed countries (1). Low-density lipoprotein (LDL) seems to be the major target of oxidative modification, making it

particularly atherogenic (2). Identification of factors protecting against oxidative modification of LDL are therefore of major interest. High-density lipoprotein (HDL) has been shown to have antioxidative potential; however, the mechanism(s) of its action is not known (3). One mechanism might be the enzymatic removal of lipid peroxides accumulating on the LDL particle by enzymes present on HDL (4). Paraoxonase (PON) is a $Ca^{+2}$ -dependent ester hydrolase that is exclusively bound to HDL in human plasma and has been shown to reduce the accumulation of lipid oxidation products on LDL (5), thus preventing the transformation of LDL into atherogenic particles (6). The human serum paraoxonase is a 43- to 45-kD protein. Its gene is located at $q^{21}$ to $q^{22}$ on the long arm of chromosome 7 (7). The amino acid sequence of paraoxonase is highly conserved among animal species, suggesting an important metabolic role for this enzyme (8). The ability of paraoxonase to detoxify organophosphorous compounds has been known for years. Its activity was determined earlier by the use of paraoxon, a widely used pesticide. The physiological substrate of paraoxonase is yet unknown (9). Watson *et al.* reported recently that oxidized phospholipids may represent a potential candidate (10). The suspected role of paraoxonase in the protection of LDL against oxidative modification, and the positive association found between paraoxonase genotypes and coronary artery disease, prompted us to investigate the effect of promoter polymorphism T (–107)C on CAD in Iranian patients.

# MATERIALS AND METHODS

A total of 300 Iranian subjects were investigated. Overall, 150 subjects were classified as having CAD (at least one coronary vessel with > 50% stenosis; $CAD^+$) and 150 subjects were classified as having negative coronary angiograms (< 20% stenosis; $CAD^-$). A structured questionnaire was used to characterize both patients ($CAD^+$) and population controls ($CAD^-$). Data collection included height and weight (to assess body mass index, BMI), age, diabetes mellitus and smoking.

Total Cholesterol and triglyceride concentrations in plasma were measured by automated enzymatic methods (Hitachi 902 Auto Analyzer). HDL cholesterol was determined in the whole plasma after precipitation of apolipoprotein B (apoB)-containing lipoproteins with phosphotungstic acid/MgCl2. LDL cholesterol was estimated according to direct method (11).

Blood was collected in 10 mL Na-EDTA tubes and kept frozen at –20 ºC. DNA was extracted by the Phenol-Proteinas K method. Promoter PON1 T (–107) C genotypes were detected by a combination of polymerase chain reaction (PCR) and digestion with restriction enzyme with some modifications. PCR amplification was performed using the following primers:

5'GACCGCAAGCCACGCCTTCTGTGCACC 3' and

5'TATATTTAATTGCAGCCGCAGCCCTGCTGG GGCAGCGCCGATTGGCCCGCCGC 3'. The PCR product was digested overnight with 5U BSTU1 restriction enzyme and diagnosed in a 4% agarose gels as undigested 119-bp fragment (mutated, T allele) or as digested 52-bp and 67-bp fragments (wild-type, C allele)(12) . The PCR mixture contained 100–200 ng DNA template, 0.2 mmol:l dNTPs, 1.5 mmol:l MgCl2, 0.3mmol:l of each primer, and 1.25 IU Taq DNA polymerase with 5% dimethyl sulfoxide (DMSO) in a final volume of 25µL.

Amplification conditions :After initial denaturation (3 min at 95°C), 35 cycle were run of 1 min at 95°C, 1 min at 63°C and 1 min at 72°C with a final extension time of 8 min (13).

We used the statistical package for social sciences (SPSS) for data analysis. A value of $P < 0.05$ was considered significant. To compare CAD patients and subjects without CAD by quantitative data (age, body mass index (BMI) and lipid levels), we used *t* test. Differences with respect to qualitative data (gender, smoking and diabetes) were analyzed using the $\chi^2$ test (Chi-square test). The $\chi^2$ test was also used to compare paraoxonase-1 genotype distributions and allele frequencies between the studied groups. Fisher's exact test was used to compare frequencies of specific allele carriers between the groups.

**Table 1.** Baseline characteristics of Subjects with or without CAD

| Variable | $CAD^+$ (n= 150) | $CAD^-$ (n= 150) | P value |
|---|---|---|---|
| Age (y) | 57.76±10.1 | 53.77±8.3 | <0.001 |
| BMI (kg/m$^2$) | 26.7±2.1 | 25.9±2.0 | 0.003 |
| Sex male (%) | 111 (74%) | 79 (52.7%) | <0.001 |
| Diabetes mellitus (%) | 30 (20%) | 7 (4.7%) | <0.001 |
| Smoking (%) | 54 (36%) | 39 (26%) | 0.061 |
| Total Cholesterol (mg/dl) | 178.61±40.3 | 181.30±162.2 | 0.844 |
| Triglycerides (mg/dl) | 161.89±102.2 | 143.24±67.2 | 0.063 |
| LDL Cholesterol (mg/dl) | 102.38±23.6 | 95.69±23.7 | 0.015 |
| HDL Cholesterol (mg/dl) | 52.89±14.1 | 54.26±13.6 | 0.392 |

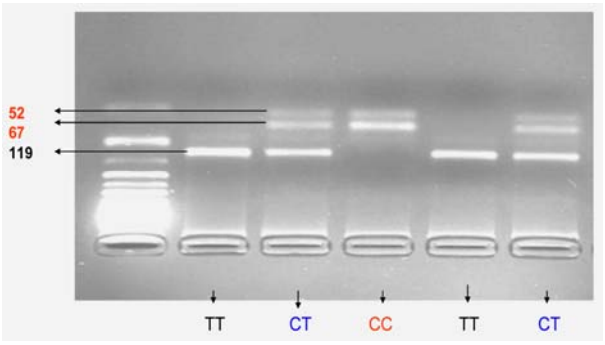


**Fig. 1.** Restriction patterns of the PCR-amplified 119-bp fragment after digestion with BSTU1 Fragment sizes of 52 bp and 67 bp correspond to C allele, whereas 119 bp are diagnostic bands for T allele.

One-way analysis of variance (ANOVA) was used to analyze relationship between genotypes and clinical and laboratory findings in the patient group (14).

## RESULTS

A total of 150 controls and 150 case subjects were included in the study.Table1 shows the clinical and biochemical characteristics of $CAD^+$ and $CAD^-$ groups. No differences were found in smoking between the groups, However $CAD^+$ subjects were older ($P$ = 0.000). $CAD^+$ subjects showed significantly higher levels of risk factors, such as male sex, diabetes, BMI and LDL-C as compared to $CAD^-$ group. No differences in total cholesterol, HDL-C and triglycerides were observed between groups.

The restriction patterns indicating the presence of three genotypes due to the existence of two common alleles, (T and C) are shown in Figure 1. The genotype distributions and allele frequencies of the studied Polymorphism (-107 T/C promoter PON1) is given in Table 2. The genotype frequencies did not differ significantly between two groups ($P$ = 0.075). The cases had a significantly higher frequency of the TT genotype compared with controls (OR=1.855 [CI 95% 1.067-3.224] $P$ = 0.027). The frequency of the T allele was significantly higher ($P$ = 0.041) in cases than in controls. When clinical and laboratory values were compared among genotype in the patient group ($CAD^+$ ) no significant differences were noted with regard to body mass index, male sex, smoking, diabetes Or triglyceride, HDL-cholesterol and LDL-cholesterol levels (Table 3).

**Table 2.** Genotype and allele frequency of Subjects with or without CAD

| Genotype | $CAD^+$ (n= 150) | $CAD^-$ (n= 150) | *P* value | OR (%95 Cl) |
|---|---|---|---|---|
| TT | 42 (28%) | 26 (17.3%) | 0.027 | 1.855 |
| CG | 76 (50.7%) | 83 (55.3%) | 0.418 | 0.829 |
| CC | 32 (21.3%) | 41 (27.3%) | 0.226 | 0.721 |
| Allele frequency | | | | |
| T | 160 (53%) | 135 (45%) | | 1.397 |

**Table 3.** Classical coronary risk factors and lipid profile of patients with CAD

| variable | T/T (n= 42) | C/T (n= 76) | C/C (n= 32) | *P* value |
|---|---|---|---|---|
| Age (y) | 53.83 ± 8.4 | 59.20 ± 11.0 | 59.50 ± 8.7 | 0.012 |
| BMI (kg/m$^2$) | 26.85 ±1.9 | 26.69 ± 2.1 | 26.52 ± 2.2 | 0.802 |
| Sex male (%) | 29 (69%) | 57 (75%) | 25 (78.1%) | 0.651 |
| Diabetes mellitus (%) | 10 (23.8%) | 11 (14.5%) | 9 (28.1%) | 0.207 |
| Smoking (%) | 11 (26.2%) | 27 (35.5%) | 16 (50%) | 0.106 |
| Cholesterol (mg/dl) | 174.8 ±39.9 | 180.2 ± 37.6 | 179.7 ± 47.3 | 0.777 |
| Triglycerides (mg/dl) | 154.3 ± 64.1 | 162.6 ± 121.5 | 170.0 ± 94.6 | 0.808 |
| LDL Cholesterol (mg/dl) | 103.0 ± 23.4 | 103.8 ± 23.6 | 97.9 ± 24.2 | 0.493 |
| HDL Cholesterol (mg/dl) | 51.23 ± 12.8 | 53.82 ± 13.5 | 52.84 ± 16.9 | 0.636 |

Abbreviations: BMI, body mass index; LDL, ; HDL,

The homozygote (TT) group was significantly younger than either CC or CT group ($P$ = 0.012). The genotype frequencies did not differ significantly ($P$ = 0.973) between one, two and three Diseased Vessels Groups (Table 4).

# DISCUSSION

Oxidative modification of LDL is known to play a pivotal role increasing the atherosclerotic risk (15). Oxidized phospholipids and cholesteryl esters are presumably the main substrates for PON1 in its protective effect against oxidation of LDL (16). Polymorphism in gene encoding for enzyme involved in modulation of LDL oxidation provides a potentially powerful approach for population studies (17). Paraoxonase genes represent good candidates, as suggested by animal models. Paraoxonase-1 knockout mice are susceptible to atherosclerosis (18). PON1 serum levels and activity are under genetic regulation, implicating the PON1 gene in the development of atherosclerosis (19). Two PON1 gene polymorphisms, L55M and Q192R, has been investigated as risk factors for CAD, but no conclusions can be drawn about their role in predicting CAD risk (20). More recently, the PON1 promoter polymorphism (-107) T/C has been identified as an independent predictor of coronary atherosclerosis (21). Therefore, we evaluated the contribution of PON1 (-107) T/C alleles to the presence of CAD in Iranian subjects.

Our results were consistent with those of Martinelli's, but different in term of TG, TC, HDL-C measures. This inconsistency would result from the sample size, where in Martinelli's study the sample size was 890 individuals and all the variables (risk factors) were shown to differ significantly (22). There are only few papers to compare the results of the genotype distribution of the-107 region of the paraoxonase prometer. Campo (2004 a, b) published two papers concerning the association of-107 region polymorphism of paraoxonase promoter with its activity and the HDL-C level in Cecil population (23).

**Table 4.** Promoter PON1 (-107)T>C polymorphism by number of diseased vessels

| | 1 (n= 65) | 2 (n= 44) | 3 (n= 41) |
|---|---|---|---|
| **Genotype** | | | |
| TT | 18 (27.7%) | 11 (25%) | 13 (31.7%) |
| CT | 33 (50.8%) | 23 (52.3%) | 20 (48.8%) |
| CC | 14 (21.5%) | 10 (22.7%) | 8 (19.5%) |
| **Allele frequency** | | | |
| T allele | 69 (53%) | 45 (51%) | 46 (56%) |

It seems as if their results were spurious since neither the primers nor the endonuclease used in their studies are applicable to the study of the pertinent polymorphism and thus their results would be unreliable. James *et al.* concluded that the ration of T/C– 107 allele, residing on the promoter of paraoxonase, was higher significantly in the $CAD^+$ diabetic patients as compared with that in the $CAD^-$ diabetic patients (24). Our study results show that the difference of T/C allele ratio was statistically significant between $CAD^+$ and $CAD^-$ groups. James showed that TT genotype was higher significantly in the $CAD^+$ diabetic patients as compared with that in the $CAD^-$ diabetic patients. Also our results show that TT genotype was higher significantly between $CAD^+$ and $CAD^-$ groups. James data introduce the TT genotype as the risk factor, consistent to our own results. Apparently information regarding the polymorphism of –107 region of the promoter seems to be useful for the risk assessment of CAD patients.

## Acknowledgments

This work was supported by grant from Tehran University of Medical Sciences.

## Conflict of interests

The authors declare that they have no competing interests.

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