Original Article

Association between Serum Paraoxonase 1 Activities (PONase/AREase) and *L55M* Polymorphism in Risk of Female Infertility

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Abstract

Background: The risk of developing female infertility has been associated with gene polymorphisms that decrease the activity of enzymes involved in systemic Oxidative Stress (OS). In this study, *PON1 L55M* polymorphism for association with susceptibility to infertility was investigated among Iranian female population.

Methods: Samples from 120 Iranian females [20 endometriosis; 30 Polycystic Ovary Syndrome (PCO); 70 controls] were analyzed and PCR-RFLP assay was used to determine the PON1 rs854560 (L55M) frequencies. The paraoxonase (PONase) and arilesterase (AREase) activities of PON1 enzyme were also assessed in order to investigate the association between serum PON1 activities, female infertility, and *PON1 L55M* polymorphism.

Results: The women with a MM genotype (p=0.021; OR=2.55) showed more possibilities of experiencing infertility than those with a LM genotype (p=0.039; OR=1.91). According to LSD test, endometriosis subjects had significantly lower paraoxonase enzyme activity compared to control group (p=0.0024; CI=95%). No significant difference was found in women with PCOS for both PONase and AREase activity in comparison with control group (p=0.469; CI=95%). Furthermore, PON1 activities were the highest in LL genotype followed by LM and then MM genotype (MM<LM<LL) in both patients and controls.

Conclusion: *PON1 L55M* polymorphism may be associated with serum PON1 activity and the risk of developing female infertility.

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Introduction

Female infertility is a complex disorder which may be caused by medical conditions including pelvic inflammatory disease, endometriosis, Polycystic Ovary Syndrome (PCOS), premature ovarian failure and uterine fibroids [1]. PCOS is a heterogeneous female endocrine metabolic disorder affecting 5-10% of women characterized by its significantly complex clinical alignments [2]. Endometriosis is a chronic, benign, estrogen-dependent condition characterized by the presence of endometrial tissue outside the uterus cavity associated with pelvic pain and infertility. The pathophysiologic mechanism of endometriosis is unknown and it affects 3-10% of women in reproductive years of their life and 20-50% of women with infertility [3-5]. In fact, PCOS and endometriosis appear to have a complex and multifactorial etiology in which a variety of genes interact with environmental factors to produce these conditions. Recent biochemical and genetic studies on the pathogenesis of PCOS and endometriosis were focused on the Single Nucleotide Polymorphisms (SNPs) affecting Oxidative Stress (OS) [6,7]. The results of these studies suggest that genetic polymorphisms in antioxidant genes including the SNPs affecting the activities of paraoxonase 1 (PON1) may contribute to female infertility [8]. Serum paraoxonase 1 is an antioxidant calcium-dependent esterase/lactonase, which circulates within High Density Lipoprotein (HDL) particles [9,10]. PON1 possesses antioxidant, anti-atherogenic, and anti-inflammatory properties and is involved in hydrolysis of several organophosphorus insecticides and nerve agents, inhibition of Low Density Lipoprotein (LDL) oxidation and increase of macrophage-associated cholesterol efflux [11]. The activities of PON1 are genetically regulated and the *PON1* gene polymorphisms have potent influences on its activities. PON1 is a member of a multigene cluster including *PON1*, *PON2*, and *PON3* [12]. Among all the SNPs of *PON1* gene, -909G/C [rs854572], -162A/G [rs705381], -108C/T [rs705379] in the promoter region and Q192R [rs662], L55M [rs-854560] in the coding region are the most studied poly-morphisms [13,14]. The L55M polymorphism influences the paraoxonase and arylesterase

(ARE) activities and the stability of the protein [15]. In this case-control study, PONase and AREase activities were examined and the association of coding L55M polymorphism with female infertility in Iranian population was investigated. The L55M polymorphism could be a risk factor in determining susceptibility to female infertility.

Materials and Methods

Subjects

This case-control study analyzed a group of 50 infertile females including 20 with endometriosis who were diagnosed by laparoscopy and 30 with PCOS fulfilling the criteria for PCOS [16], who attended the Isfahan Fertility-Infertility Center and Royan Institute. The control group consisted of 70 healthy age-matched volunteers who visited the clinic for a regular health check-up with proven fertility and no clinical or biochemical hyperandrogenism, no menstrual cycle irregularities, and no history of endometriosis and PCOS. Women from different regions of Iran were referred to this infertility center for check-up and they made the population of this study. There was no evidence of known paraoxonase-affecting diseases such as CHD, liver diseases, atherosclerosis, diabetes, hypertension, cancer and infections in all fertile and infertile females who were not genetically related. Blood sampling was done based on patient consent and an agreement was signed between the University of Isfahan and Isfahan Fertility-Infertility Center. Blood samples were obtained in the morning after overnight fasting and collected in EDTA and heparin coated tubes to analyze *PON1 L55M* polymorphism and PON1 activities. Blood and serum samples were stored at -20°*C* until analysis.

Genotyping analyses

For genotype analysis, genomic DNA was extracted from peripheral blood using a salting-out method described by Miller [17] and was stored at -20°*C* till genotyping could be performed. Coding L55M (rs 854560) genotyping was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers for SNP were designed using the primer design software Oligo 7 and were listed as follows:

Forward primer: 5′ TGAATTATTCTGAACCTATTA AAGAAGA 3′

Reverse primer: 5′ AAGACTTAAACTGCCAGTCCT AGA 3′

PCR was performed in 25 *µl* reaction mixture containing 2 *µl* of each forward and reverse primers (10 *pM*), 2.5 *µl* of ×10 solution buffer (20 *mM* Tris-HCl pH=8.6, 50 *mM* KCl, Cinnagen Inc, Iran), 0.5 *µl* of four mixed dNTPs (10 *mM*, Cinnagen Inc, Iran), 0.75 *µl* of Mgcl2 (50 *mM*, Cinnagen Inc, Iran), 0.3 *µl* of 5*u/µl* Taq DNA polymerase (Cinnagene, Co., Iran), and 2 *µl* (100 *ng/µl*) of genomic DNA. The PCR program was as follows: initial denaturation for 4 *min* at 94°*C* followed by 35 cycles of 40 *s* at 94°*C*, 35 *s* at 54-66°*C*, 72°*C* for 40 *s*, and a final extension step of 15 *min* at 72°*C*. The PCR products were run on 2% agarose gel and were visualized by ethidium bromide staining. Moreover, a restriction analysis was performed using 10 units of *Hin*1II enzyme (Fermentas, Vilnius, Lithuania) in buffer G (Fermentas) at 37°*C* for 16 *hr*. The restriction fragments were analyzed on 2% agarose gels and were visualized through ethidium bromide staining. Digested DNA fragments of 146 and 103 *bp* were detected in MM homozygotes (restriction site present), an undigested band of 249 *bp* was detected in LL homozygotes (restriction site absent) and heterozygous genotypes resulted in three different bands (146, 103 and 249 *bp*) through PCR-RFLP technique (Figure 1).

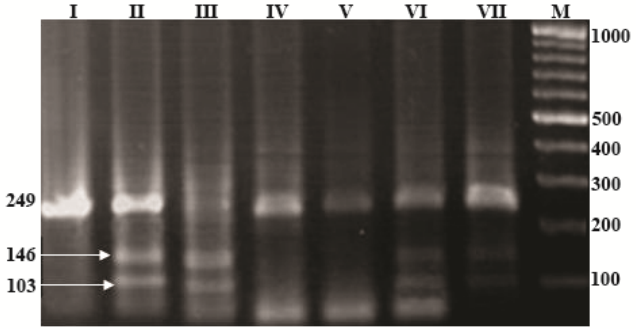

Figure 1. Electrophoresis pattern of *PON1 L55M* polymorphism using PCR-RFLP and *Hin*1II restriction enzyme. I) Undigested PCR products, II) LM (103,146, and 249 *bp* fragments). III to VII) MM (103, and 146 *bp* fragments), LL (249 *bp* fragment), LL, and LM genotype, respectively. M) 100 *bp* ladder. Numbers are in base pair (*pb*).

Analyses of PON1 activities

The serum was isolated by centrifugation at 800 *g* for 10 *min* at 4°*C* and was immediately frozen at -80°*C* in order to determine PON1 activities in blood samples. The PONase activity was measured using paraoxon (Sigma Chem, USA) as substrate and the increase in absorbance at 412 *nm* was determined, because of 4 -nitrophenol formation. The activity was measured at 25°*C* during 3 *min* after 5 *µl* of serum were added to each well containing 100 *µl* of Tris/HCl (100 *mMol*, pH=8.0) buffer including 2 *mMol* $CaCl_2$ and 5.5 *mMol* of paraoxon. All results are expressed in *U/ml* which is defined as 1 *nmol* of 4- nitrophenol formed per minute. Arilesterase activity in serum was determined spectrophotometrically using the phenylacetate (Merck-Schardt) as a synthetic substrate. The reaction started by adding 100 *µl* of phenylacetate (10 *mM*) as substrate solution to wells containing 5 *µl* of serum (prediluted 1:10) and 1 *mM* $CaCl_2$ (Sigma, USA) in 50 *mM* Tris buffer pH=8. The phenol production was measured during 2 *min* at 270 *nm* and pH=8.0. The PON1's arilesterase activity is expressed as *KU/ml* which is defined as 1 *µmol* arilesterase per minute. PON1's paraoxonase and arilesterase activities were determined in triplicate [18].

Table 1. Association between *PON1 L55M* polymorphism and female infertility

1st Polymorphism	2nd Polymorphism	1st and 2nd mean difference	Standard deviation	p-value
MM				
	LM	0.23214 *	0.09928	0.021
	LL	-0.02381	0.12924	0.854
LM				
	MM	-0.23214 *	0.09928	0.021
	LL	-0.25595 *	0.12272	0.039
LL				
	MM	-0.02381	0.09928	0.854
	LM	0.25595 *	0.12924	0.039

* Significance difference with 95% confidence interval.

Table 2. Characteristics of cases and control samples (mean±SD)

	PCOS	Endometriosis	Fertile
Age	31.92±4.38	31.54±6.212	32.09±6.56
Body Mass Index (BMI)	28.03±5.41	24.89±4.02	24.66±3.86
Paraoxonase activity (*U/l*)	1682.88±2255.47	1259.90±1680.95	2529.02±3144.78
Arilesterase activity (*KU/l*)	227.67±131.11	212.88±119.79	255.58±145.53
Serum PON1 activity	7.39±6.89	5.91±6.78	9.89±5.71

Briefly, serum PON1 activity (PON1 phenotype) was determined by double substrate method. Phenotype is defined as the activity ratio of PONase activity divided by AREase activity.

Statistical analyses

Statistical analyses were performed using SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA) software. The Chi-square distribution (χ^2) was used to evaluate the PON1 genotype frequencies between the patient and the control groups. Multiple comparisons were done by employing one way analysis of variance (ANOVA) followed by a LSD (least significant difference) test. All data are presented as means ± standard deviation (SD). Statistical significance was assessed using the 5% significance level.

Results

Distribution of PON1 L55M genotype

The association between *PON1 L55M* polymorphism and female infertility in case and control subjects is shown in table 1. The distribution of genotypes were in Hardy-Weinberg equilibrium (p=0.45). The results showed that there were significant differences regarding *PON1 L55M* polymorphism among fertile and infertile groups. The women with a MM genotype (p=0.021; OR=2.55) were statistically associated with infertility in comparison to those with a LM genotype (p=0.039; OR=1.91). There was no association between L55M polymorphism and each infertile group including patients with endometriosis and PCOS separately.

PONase, AREase activities and infertility

The group mean age, body mass index (BMI), and the serum PON1's paraoxonase and arilesterase activities are presented in table 2 for fertile, PCOs, and endometriosis groups. Paraoxonase enzyme activity was highly variable among different samples. According to LSD test, endometriosis subjects had significantly lower paraoxonase enzyme activity compared to control groups (p=0.0024; CI=95%) (Table 3). No significant difference was found in women with PCOS for both PONase and AREase activity in comparison with control group (p=0.469; CI=95%).

Table 3. Association between infertility and paraoxonase enzyme activity

Group 1	Group 2	Mean difference between two groups	Standard deviation	p-value
Endometriosis				
	Fertile	-3.86913 *	1.68803	0.024
	PCO	-1.14816	1.58038	0.469
Fertile				
	Endometriosis	4.86913 *	1.68803	0.024
	PCO	2.72097	2.01152	0.179
PCO				
	Endometriosis	1.14816	1.58038	0.469
	Fertile	-2.72097	2.01157	0.179

* Significance difference with 95% confidence interval.

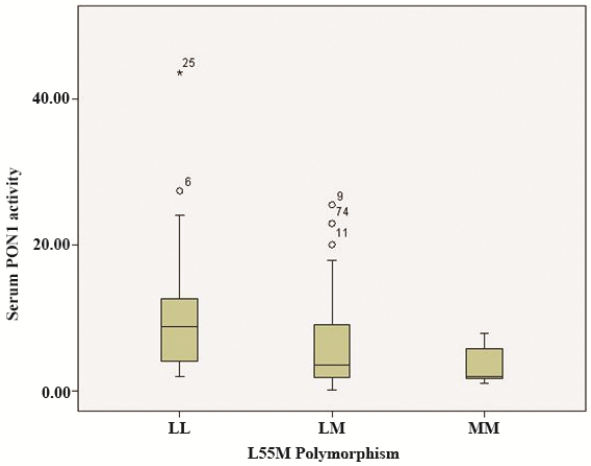

Figure 2. L55M genotypes (LL, LM and MM) and serum PON1 activity in both controls and patients. Serum PON1 activity was the highest in LL genotype followed by LM and then MM genotype (MM<LM<LL) in both patients and controls.

Association between PON1 L55M polymorphism and serum PON1 activities

The association of *PON1 L55M* polymorphism with PON1 activities is shown in figure 2. Significant difference was observed among the genotypes of L55M polymorphism in patient and control groups. Serum PON1 activity was the highest in LL genotype followed by LM and then MM genotype (MM<LM<LL) in both patients and controls. It was found that in three genotypes, patients had significantly lower activity in comparison with controls.

Discussion

Serum PON1 is a esterase/lactonase which has significant roles in protection of LDL and HDL against oxidation [19-23]. Recently, PON1 genotyping and analysis of serum PON1 activity has been comprehensively studied to investigate associations with variety of diseases including cardiovascular disease, diabetes, infertility, Alzheimer, cancer, and Parkinson [20-24]. Genetic variants in *PON1* gene may modulate OS and thus affect susceptibility to female infertility. OS which was defined as imbalance between pro-oxidants and antioxidants has critical roles in normal functioning of the female reproductive system and development of female reproductive diseases such as endometriosis, and PCOS [25,26]. The effects of OS on male infertility have been well described, but its impacts on female reproductive disorders are generally unknown. In this case-control study, PON1 enzyme activities (PONase and AREase), and *PON1 L55M* coding polymorphism were investigated in Iranian infertile women population for the first time. The global minor allele frequency of *PON1 L55M* polymorphism (rs854560) is T=0.1827/915 according to the dSNP (www.ncbi.nlm.nih.gov/snp). This means that for rs854560, minor allele is 'T' and has a frequency of 18.27% in the 1000 genome phase 1 population. It was found that serum PON1 activity was significantly lower in endometriosis patients in comparison with controls. This result is similar to previous studies which have been performed in different populations [27,28]. In another study, Verit *et al* reported a significant difference in two groups of endometriosis patients including women with moderate to severe endometriosis and women with minimal to mild endometriosis. PON-1 activity was significantly lower in women with moderate to severe endometriosis than in women with minimal to mild endometriosis and controls ($p<0.0001$) [29]. The association between PON1 activity and PCOS was suggested by Dursun *et al* for the first time. They reported a significant difference ($p=0.027$) in PON1 activity between PCOS patients and control groups [30]. In the present study, there was no significant difference in serum PON1 activity of PCOS patients in comparison with the control group. The association of *PON1 L55M* polymorphism and PON1 activity was also evaluated in our study which indicated that PON1 activity was affected by L55M polymorphism. PONase activity was the highest in LL genotype and lowest in MM genotype in both patients and control groups ($p=0.002$; CI=95%) and in all the three genotypes (LL, LM, MM); patients had lower PONase activity in comparison with controls. PON's activity varies widely between individuals and is related to several other environmental factors. Our further analyses indicate that AREase activity was independent of L55M polymorphism which is similar to the observation by Chen *et al* [31]. Briefly, serum PON1 activity or PON1 phenotype was the highest in LL genotype followed by LM and then MM genotype (MM<LM<LL) in both patients and controls. In addition, it was found that MM genotype was more common in infertile females as compared to the controls (OR=2.55; $p=0.021$; χ^2 significance=0.029) and LM genotype was associated with low risk of infertility (OR=1.91; $p=0.039$) (Table 4). Furthermore, it was observed that the frequencies of *PON1 L55M* polymorphism in this Iranian population were similar to those for European Caucasians but different from those seen in other Asian populations [32]. The findings of a case-control study which was performed by Sepehrvand *et al* confirmed this similarity of *PON1 L55M* polymorphism frequency in other Iranian population [33,34].

Conclusion

In conclusion, results of this study support the hy-

Table 4. Distribution of *PON1 L55M* genotypes in fertile and infertile groups

Genotype	Fertile, N[a] (f)	Infertile, N (f[b])	OR[c] (CI[d] 95%)	p-value
LL	20 (28.6)	20 (40)		
LM	40 (57.2)	15 (30)	1.91	0.039
MM	10 (14.28)	15 (30)	2.55	0.021
Total	70	50		

a: Number; b: Frequency; c: Odd ratio.

pothesis that *PON1 L55M* polymorphism may have significant roles in serum PON1 activity and risk of developing female infertility. Our findings suggest further analyses are required to investigate other PON1 polymorphisms and their importance on the individual's susceptibility to infertility in larger groups.

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Conflict of Interest

All authors have seen and agreed with the contents of the manuscript and there is no conflict of interest to report.

References

1. Gnoth C, Godehardt E, Frank-Herrmann P, Friol K, Tigges Jr, Freundl G. Definition and prevalence of subfertility and infertility. Hum Reprod 2005;20(5):1144-1147.
2. Deligeoroglou E, Kouskouti C, Christopoulos P. The role of genes in the polycystic ovary syndrome: predisposition and mechanisms. Gynecol Endocrinol 2009;25(9): 603-609.
3. Richter RJ, Jarvik GP, Furlong CE. Paraoxonase 1 status as a risk factor for disease or exposure. Adv Med Biol 2010;660:29-35.
4. Bragatto FB, Barbosa CP, Christofolini DM, Peluso C, dos Santos AA, Mafra FA, et al. There is no relationship between Paraoxonase serum level activity in women with endometriosis and the stage of the disease: an observational study. Reprod Health 2013;10:32.
5. Augoulea A, Alexandrou A, Creatsa M, Vrachnis N, Lambrinoudaki I. Pathogenesis of endometriosis: the role of genetics, inflammation and oxidative stress. Arch Gynecol Obstet 2012;286(1):99-103.
6. Ji G, Gu A, Wang Y, Huang C, Hu F, Zhou Y, et al. Genetic variants in antioxidant genes are associated with sperm DNA damage and risk of male infertility in a Chinese population. Free Rad Biol Med 2012;52(4):775-780.
7. Yeon Lee J, Baw C-K, Gupta S, Aziz N, Agarwal A. Role of oxidative stress in polycystic ovary syndrome. Curr Womens Health Rev 2010;6(2):96-107.
8. H Sekhon L, Gupta S, Kim Y, Agarwal A. Female infertility and antioxidants. Curr Womens Health Rev 2010;6 (2):84-95.
9. Goswami B, Tayal D, Gupta N, Mallika V. Paraoxonase: a multifaceted biomolecule. Clin Chim Acta 2009;410 (1):1-12.
10. bin Ali A, Zhang Q, Lim YK, Fang D, Retnam L, Lim SK. Expression of major HDL-associated antioxidant PON-1 is gender dependent and regulated during inflammation. Free Radic Biol Med 2003;34(7):824-829.
11. Camps J, Marsillach J, Joven J. The paraoxonases: role in human diseases and methodological difficulties in measurement. Crit Rev Lab Sci 2009;46(2):83-106.
12. Précourt LP, Amre D, Denis MC, Lavoie JC, Delvin E, Seidman E, et al. The three-gene paraoxonase family: physiologic roles, actions and regulation. Atherosclerosis 2011;214(1):20-36.
13. Kokouva M, Koureas M, Dardiotis E, Almpanidou P, Kalogeraki A, Kyriakou D, et al. Relationship between the paraoxonase 1 (PON1) M55L and Q192R polymorphisms and lymphohaematopoietic cancers in a Greek agricultural population. Toxicology 2013;307:12-16.
14. Ferk P, Gersak K. Association of -108 C>T PON1 polymorphism with polycystic ovary syndrome. Biomed Rep 2014;2(2):255-259.
15. Abu Farha M, Fawzy M, Abdel Wahab A, Rashed L, Farouq A. Relation between paraoxonase 1 (PON1) gene polymorphism insulin resistance in polycystic ovary syndrome (PCOS). Kasr Al-Aini J Obstet & Gynecol 2011;1(2):75-82.
16. Homburg R. The management of infertility associated with polycystic ovary syndrome. Reprod Biol Endocrinol 2003;1:109.
17. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16(3):1215.
18. Sumegova K, Nagyova Z, Waczulikova I, Zitnanova I, Durackova Z. Activity of paraoxonase 1 and lipid profile in healthy children. Physiol Res 2007;56(3):351-357.
19. Augoulea A, Mastorakos G, Lambrinoudaki I, Christodoulakos G, Creatsas G. The role of the oxidative-stress in the endometriosis-related infertility. Gynecol Endocrinol 2009;25(2):75-81.
20. Gur M, Aslan M, Yildiz A, Demirbag R, Yilmaz R, Selek S, et al. Paraoxonase and arylesterase activities in coronary artery disease. Eur J Clin Invest 2006;36(11): 779-787.
21. Marsillach J, Camps J, Beltran-Debon R, Rull A, Aragones G, Maestre Martnez C, et al. Immunohistochemical analysis of paraoxonases-1 and 3 in human atheromatous plaques. Eur J Clin Invest 2011;41(3):308-314.
22. Ozenoglu A, Balci H, Ugurlu S, Caglar E, Uzun H, Sarkis C, et al. The relationships of leptin, adiponectin levels and paraoxonase activity with metabolic and cardiovascular risk factors in females treated with psychiatric drugs. Clinics (Sao Paulo) 2008;63(5):651-660.
23. Ikeda Y, Suehiro T, Inoue M, Nakauchi Y, Morita T, Arii K, et al. Serum paraoxonase activity and its relationship to diabetic complications in patients with non-insulin-dependent diabetes mellitus. Metabolism 1998;47(5): 598-602.
24. Elkiran ET, Mar N, Aygen B, Gursu F, Karaoglu A, Koca S. Serum paraoxonase and arylesterase activities in patients with lung cancer in a Turkish population. BMC cancer 2007;7(1):48.
25. Ginsberg G, Neafsey P, Hattis D, Guyton KZ, Johns DO, Sonawane B. Genetic polymorphism in paraoxonase 1

(PON1): Population distribution of PON1 activity. J Toxicol Environ Health B Crit Rev 2009;12(5-6):473-507.

26. Agarwal A, Gupta S, Sharma R. Oxidative stress and its implications in female infertility-a clinician's perspective. Reprod Biomed Online 2005;11(5):641-650.
27. Jackson LW, Schisterman EF, Dey-Rao R, Browne R, Armstrong D. Oxidative stress and endometriosis. Hum Reprod 2005;20(7):2014-2020.
28. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol 2005;3:28.
29. Verit FF, Erel O, Celik N. Serum paraoxonase-1 activity in women with endometriosis and its relationship with the stage of the disease. Hum Reprod 2008;23(1):100-104.
30. Dursun P, Demirtaş E, Bayrak A, Yarali H. Decreased serum paraoxonase 1 (PON1) activity: an additional risk factor for atherosclerotic heart disease in patients with PCOS? Hum Reprod 2006;21(1):104-108.
31. Chen Q, Reis SE, Kammerer CM, McNamara DM, Holubkov R, Sharaf BL, et al. Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation (WISE) study. Am J Hum Genet 2003;72(1):13-22.
32. Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE. Effects of 5' regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. Am J Hum Genet 2001;68(6):1428-1436.
33. Sepahvand F, Rahimi-Moghaddam P, Shafiei M, Ghaffari SM, Rostam-Shirazi M, Mahmoudian M. Frequency of paraoxonase 192/55 polymorphism in an Iranian population. J Toxicol Environ Health A 2007;70(13):1125-1129.
34. Imai Y, Morita H, Kurihara H, Sugiyama T, Kato N, Ebihara A, et al. Evidence for association between paraoxonase gene polymorphisms and atherosclerotic diseases. Atherosclerosis 2000;149(2):435-442.