Environ Health Prev Med (2012) 17:316–321
DOI 10.1007/s12199-011-0256-4

REGULAR ARTICLE

Effect of cigarette smoking on paraoxonase 1 activity according to PON1 L55M and PON1 Q192R gene polymorphisms

Dhouha Haj Mouhamed · Asma Ezzaher · Anwar Mechri · Fadoua Neffati · Asma Omezzine · Ali Bouslama · Lotfi Gaha · Wahiba Douki · Mohamed Fadhel Najjar

Received: 23 September 2011 / Accepted: 26 November 2011 / Published online: 29 December 2011

Abstract
Objective This study aims to investigate the effect of cigarette smoking on paraoxonase 1 (PON1) activity according to PON1 L55M and PON1 Q192R gene polymorphisms.
Materials and methods Our sample included 300 voluntary subjects: 138 nonsmokers and 162 current smokers aged 38.47 ± 21.91 and 35.55 ± 16.03 years, respectively. PON1 activity was determined by kinetic methods. L55M and Q192R gene polymorphisms of PON1 were determined by multiplex polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP).
Results We found in smokers a significant decrease of PON1 activity before and after adjustment. We noted a significant association between smoking status and lower PON1 activity [odds ratio (OR) = 3.03, confidence interval 95% = 1.5–5.9, $p = 0.001$]. In smokers, there was significant association between PON1 activity and PON1 L55M polymorphisms ($p = 0.01$). Also, the 55MM genotype presented the lowest paraoxonase activity, while the 55LL genotype showed the highest one. After adjustment for confounding variables, smokers with PON1 L55M polymorphism had the highest risk for lower PON1 activity; however, PON1 Q192R genotype might protect smokers from decrease in PON1 activity. We found significant interaction between the effect of cigarette smoking and both PON1 L55M and PON1 Q192R polymorphisms on lower PON1 activity.
Conclusions Cigarette smoking was significantly associated with decrease in PON1 activity. Moreover, PON1 L55M polymorphism predisposes smokers to decreased PON1 activity in contrast to PON1 Q192R genotype.

Keywords Smoking · PON1 activity · PON1 L55M and PON1 Q192R gene polymorphisms

Introduction

Smoking is firmly established as 1 of the principal cardiovascular risk factors. In Tunisia, literature suggests that smoking prevalence is very high, especially among young people. Among health professionals and medical students, this prevalence is the same as in the general population [1]. Tobacco-related diseases are the leading cause of mortality among men, representing 22% of total male deaths for those older than 25 years [1]. Oxidative stress is considered to be the major pathological mechanism associated with smoking, leading notably to lipid peroxidation [2]. Several studies have demonstrated increased susceptibility of low-density lipoprotein (LDL) to oxidation and higher levels of oxidized LDL in smokers. This would provide an important causal mechanism that links smoking with vascular disease, given the numerous pathological effects of oxidized LDL. Smoking may enhance oxidative stress not only through production of reactive oxygen radicals in smoke but also through weakening of antioxidant defense mechanisms. In this

D. Haj Mouhamed (✉) · A. Ezzaher · F. Neffati · W. Douki · M. F. Najjar
Laboratory of Biochemistry-Toxicology, Monastir University Hospital, 5000 Monastir, Tunisia
e-mail: hajdhouha@yahoo.fr

D. Haj Mouhamed · A. Ezzaher · A. Mechri · L. Gaha · W. Douki
Research Laboratory "Vulnerability to Psychotic Disorders LR 05 ES 10", Department of Psychiatry, Monastir University Hospital, Monastir, Tunisia

A. Omezzine · A. Bouslama
Laboratory of Biochemistry, Sahloul University Hospital, Sousse, Tunisia

context, a recent study showed that cigarette smoke inhibited the enzymatic activity of paraoxonase (PON). Given its hypothesized antioxidant role, this could also contribute to increased oxidation of LDL in smokers [2].

PON1 (EC 3.1.8.1) is a calcium-dependent esterase that circulates in plasma associated with high-density lipoprotein (HDL) and contributes to the protective effect of this lipoprotein on LDL oxidation [2]. Some authors have extended this suggested antioxidant role of PON1 to general prevention of peroxidative damage to cell membranes. However, the enormous variation in serum PON1 activity (40-fold) observed among individuals complicates its clinical interpretation. This variation can be explained by both genetic and environmental influences [3].

Polymorphisms in the promoter and coding regions of the paraoxonase gene are the main determinants of its expression and the enzymatic activity [4, 5], but serum paraoxonase activity can be modulated by several environmental factors. Pathologic states such as renal disease, diabetes mellitus, cardiovascular disease, and liver cirrhosis are associated with decreased paraoxonase activity, and various dietary and lifestyle factors have been reported to influence serum paraoxonase activity. Tobacco smoking has been associated with reduced PON1 activity in patients with coronary artery disease [6], and extracts of cigarette smoke inhibited PON1 activity in vitro [7]. Pharmacologic therapy with simvastatin [8] and hormone replacement therapy [9], on the other hand, has been reported to increase serum PON1 activity.

The differences in paraoxonase activity among individuals are explained partly by genetic variation in the coding region of the paraoxonase gene; this variation leads to substitution of arginine for glutamine at position 192 (Q192R) and methionine for leucine at position 55 (M/L55). The M/L55 polymorphism is also a significant determinant of the serum concentration of paraoxonase [10]. Several case–control studies have shown a positive association between PON1 R192 allele and coronary heart disease, while several other studies have shown no association [11]. This study aims to investigate the effect of cigarette smoking on PON1 activity according to PON1 L55M and PON1 Q192R gene polymorphisms and the correlation between this parameter and two biological tobacco markers: plasma thiocyanate (SCN^-) and cotininuria.

Materials and methods

Study design

Population

This study was approved by the local ethical committee, and all subjects were of Tunisian origin. Our sample included 300 voluntary subjects: 138 nonsmokers (62 men and 76 women) aged 38.47 ± 21.91 years and 162 current smokers (145 men and 17 women) aged 35.55 ± 16.03 years. Subjects with peripheral vascular disease, diabetes mellitus, renal disease, hepatic disease, hyperlipidemia, or hypertension or receiving any medication were also excluded. Written informed consent was obtained from all voluntary adult participants and from the parents of minors.

Samples

After 12-h overnight fasting, venous blood from each subject was drawn in tubes containing lithium heparinate for classical biochemical parameters, and in tubes containing EDTA/K_3 for genotype analysis. Blood samples were immediately centrifuged at +4°C and stored at −80°C until biochemical analyses. Urine samples were obtained from the smokers and nonsmokers. These samples were either used the same day or frozen at −20°C until required for analysis. All the samples were analysed for urine cotinine.

Methods

Smoking questionnaire

All subjects were questioned about their age, gender, and cigarette and alcohol consumption habits. The clinical and sociodemographic characteristics are presented in Table 1. Differences between patients and controls in terms of gender, body mass index (BMI), and alcoholic beverage consumption were noted. Therefore, these variables were considered as potential confounder factors for this analysis.

Biochemical assays

Lipid profile assay

Total cholesterol (TC), HDL cholesterol (HDLc), and triglycerides (TG) were determined by enzymatic methods, and apolipoprotein (ApoA1, ApoB) and lipoprotein (a) [Lp(a)] levels were determined by immunoturbidimetric techniques using Konelab 30^{TM} equipment. Cotinine levels were determined using homogenous enzyme immunoassay method (Konelab 30^{TM}; Thermo Electron Corporation, Finland) and expressed as micrograms per micromol creatinine in urine. SCN^- levels were determined using selective electrodes (Ionometer Seven Multi S80; Mettler Toledo, Switzerland) and expressed as milligrams per liter in plasma. BMI was calculated as weight (kg) divided by height squared (m^2).

Table 1 Variations of lipid profile, two tobacco biomarkers, paraoxonase activity, and PON1 L55M and PON1 Q192R genotype frequencies according to smoking status

	Smokers ($n = 162$)	Nonsmokers ($n = 138$)	p
Age (years)	35.6 ± 16.0	38.5 ± 21.9	0.172
Sex ratio	2.87	0.69	<0.001
BMI (kg/m^2)	24.24 ± 3.17	25.63 ± 4.36	0.003
TC (mmol/L)	4.13 ± 1.18	3.70 ± 1.04	0.005
HDLc (mmol/L)	0.94 ± 0.25	1.07 ± 0.27	0.001
LDLc (mmol/L)	1.35 ± 0.56	1.16 ± 0.61	0.01
TG (mmol/L)	1.79 ± 1.03	1.40 ± 1.24	<0.0001
ApoB/ApoA$_1$	0.83 ± 0.52	0.52 ± 0.15	0.03
Lp(a) (g/L)	0.23 ± 0.23	0.18 ± 0.19	0.04
Urine cotinine (μg/μmol Cr)	231.43 ± 205.22	73.71 ± 73.22	<10^{-7}
Plasma thiocyanate (μmol/L)	100.25 ± 1.36	99.60 ± 0.91	0.0005
PON1 activity (IU/L)	94 ± 104	158 ± 133	0.001
PON1 L55M			
LL, *n* (%)	65 (40.3)	76 (55)	$\chi^2 = 7.34$; $p = 0.02$
LM, *n* (%)	66 (40.9)	46 (33.4)	
MM, *n* (%)	31 (18.8)	16 (11.6)	
PON1 Q192R			
QQ, *n* (%)	99 (61.0)	103 (74.4)	$\chi^2 = 6.83$; $p = 0.03$
QR, *n* (%)	54 (33.3)	32 (23.3)	
RR, *n* (%)	9 (5.7)	3 (2.3)	

Cr creatinine

Paraoxonase 1 activity

Paraoxonase 1 activity was determined using paraoxon (1.2 mmol/L) as substrate in 0.1 M Tris-HCl buffer at pH 8.0, containing 2 mM $CaCl_2$ (0.5 mL final volume). The sample to be tested was added (5 μL) to start the reaction, and the increase in absorbance at 405 nm was recorded [12]. One international unit (IU) of paraoxonase 1 activity is defined as 1 μmol *p*-nitrophenol formed per minute, and activity was expressed as IU/L of plasma.

Genotype analysis

Genomic DNA was extracted from venous blood by salting-out method [13]. The Q192R and L55M polymorphisms of the PON1 gene were simultaneously studied by the protocol described previously by Motti et al. [14] with some modifications. In brief, a multiplex polymerase chain reaction (PCR) method using specific primers corresponding to the PON1 nucleotide sequence was carried out, and the PCR products were digested with *Hin*fI and then electrophoresed on 4% agarose gels stained with ethidium bromide.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, USA). Hardy–Weinberg equilibrium was evaluated and genotype frequencies were compared by chi-squared test (χ^2). Quantitative variables are presented as mean ± standard deviation (SD), and comparisons were performed using Student's t test. Qualitative variable comparisons were performed using the χ^2 test. Odds ratios (ORs) and their 95% confidence interval (CI) were calculated. Differences in demographic characteristics of smokers were assessed among the PON1 Q192R and L55M genotypes using analysis of variance (ANOVA). Adjustment for potential confounder factors was performed by binary logistic regression. The statistical significance level was set at $p < 0.05$.

Results

As shown in Table 1, PON1 activity and HDLc concentration were significantly lower in smokers than in nonsmokers. Also, smokers had significantly higher levels of TC, TG, LDLc, and Lp(a) and ApoB/ApoA$_1$ ratio than nonsmokers. We found significant dissimilarity between smokers and nonsmokers with respect to urine cotinine ($p < 10^{-7}$) and plasma SCN^- concentrations ($p = 5 \times 10^{-4}$). PON1 activity had significant negative correlation with urine cotinine ($r = -0.271$; $p = 0.03$) and at the limit of the statistical significance with plasma SCN^- ($r = -0.188$; $p = 0.06$).

The genotypic distributions among smokers and nonsmokers were within Hardy–Weinberg equilibrium. Significant differences were detected in the distribution of genotype frequencies of L55M and Q192R polymorphisms ($\chi^2 = 7.34$, $df = 2$, $p = 0.02$; $\chi^2 = 6.83$, $df = 2$, $p = 0.03$, respectively) between smokers and nonsmokers (Table 1).

After adjustment of PON1 activity levels for potential confounders (lipid profile, BMI, gender, and age), we noted significant difference between smokers and nonsmokers ($p = 0.002$).

To evaluate the adjusted association between smoking status and lower paraoxonase activity, we calculated the odds ratio of lower paraoxonase activity (<90 IU/L) associated with smoking status and adjusted for confounder factors (age, gender, BMI, and lipid profile). We noted a significant association between smoking status and lower paraoxonase activity (OR = 3.03, CI = 1.5–5.9, $p = 0.001$) (Table 2).

Table 2 Odds ratio of lower paraoxonase activity associated with smoking status

	Smoking status		OR	95% CI	p	OR[a]	95% CI	p
	Smokers n (%)	Nonsmokers n (%)						
PON1 activity <90 IU/L	104 (64)	49 (35)	3.21	1.7–5.8	$<10^{-4}$	3.03	1.5–5.9	0.001

PON1 activity: 90 IU/L median

[a] Adjusted for age, gender, BMI, and lipid profile

Table 3 Effect of paraoxonase L55M polymorphism on paraoxonase activity and HDLc concentration according to smoking status

Parameter	PON1 L55M genotype	Smokers	Nonsmokers	p[d]
PON1 activity (IU/L)	LL	114 ± 111[a]	175 ± 138	0.03
	LM	93 ± 101[b]	147 ± 128	0.01
	MM	64 ± 95[a,b]	95 ± 76	0.04
p[c]		0.01	0.09	
HDLc (mmol/L)	LL	0.95 ± 0.23	1.07 ± 0.27	0.004
	LM	0.94 ± 0.22	1.1 ± 0.29	0.003
	MM	0.93 ± 0.34	0.95 ± 0.18	0.03
p[c]		0.15	0.18	

[a] PON1 activity: LL versus MM, $p = 0.005$

[b] PON1 activity: LM versus MM, $p = 0.02$

[c] ANOVA test

[d] Student's t test

Table 4 Effect of paraoxonase Q192R polymorphism on paraoxonase activity and HDLc concentration according to smoking status

Parameter	PON1 Q192R genotype	Smokers	Nonsmokers	p[c]
PON1 activity (IU/L)	QQ	41 ± 39	76 ± 39	0.03
	QR	100 ± 108	142 ± 128	0.008
	RR	107 ± 99	211 ± 144	0.04
p[b]		0.4	0.09	
HDLc (mmol/L)	QQ	0.90 ± 0.25[a]	1.00 ± 0.16	0.001
	QR	0.96 ± 0.23[a]	1.03 ± 0.33	0.04
	RR	0.98 ± 0.28	1.08 ± 0.26	0.04
p[b]		0.6	0.6	

[a] HDLc: QQ versus RR, $p < 0.05$

[b] ANOVA test

[c] Student's t test

In smokers, there was significant association between PON1 activity and PON1 L55M polymorphisms ($p = 0.01$). Also, the 55MM genotype presented the lowest paraoxonase activity, while the 55LL genotype showed the highest one. Among HDLc concentration, there was no significant association with PON1 L55M polymorphisms. Levels of paraoxonase activity and HDLc were significantly decreased in smokers compared with nonsmokers regardless of PON1 L55M polymorphisms (Table 3).

In smokers, there was no significant association between either paraoxonase activity or HDLc concentration and PON1 192 polymorphisms ($p = 0.4$). Also, the 192QQ genotype presented the lowest paraoxonase activity, while the 192RR genotype showed the highest one. Levels of PON1 activity and HDLc concentration were significantly decreased in smokers compared with nonsmokers regardless of PON1 192 polymorphisms (Table 4).

Table 5 presents the ORs of the PON1 activity-lowering effect of cigarette smoking according to two paraoxonase genes polymorphisms. After adjustment for confounding variables, smokers with PON1 L55M polymorphism had the highest risk for lower PON1 activity; however, PON1 Q192R genotype might protect smokers from decrease in PON1 activity. We found significant interaction between the effect of cigarette smoking and both PON1 L55M and PON1 Q192R polymorphisms on lower PON1 activity.

Discussion

Previous studies have demonstrated a fall in PON1 activity and HDLc concentration and a rise in TC, TG, LDLc, Lp(a), and ApoB/ApoA1 ratio in smokers. It is known that smoking is associated with coronary artery disease and other vascular disorders. For the occurrence of cardiovascular disease among smokers, alteration in plasma lipid profile has been implicated. In this context, the mechanisms for altered lipid profile in smokers are recalled [15]. First, nicotine stimulates adrenaline release from the adrenal cortex, leading to increased serum concentration of free fatty acids, which further stimulates hepatic synthesis and secretion of cholesterol as well as hepatic secretion of very low-density lipoprotein (VLDL) and hence increased TG [15]. Second, smoking decreases estrogen levels and further leads to decreased HDLc concentration [15]. Also, HDL concentration was inversely related to VLDL concentration in serum. Finally, smoking increases insulin resistance and thus causes hyperinsulinemia. LDL and TG are elevated in hyperinsulinemic conditions due to decreased activity of lipoprotein lipase. Also, human serum paraoxonase is a polymorph enzyme which has been shown

Table 5 Effect of cigarette smoking according to two paraoxonase gene polymorphisms on lower paraoxonase activity (<90 IU/L)

	Smoking status		OR	95% CI	*p*	OR[a]	95% CI	*p*
	Smokers *n* (%)	Nonsmokers *n* (%)						
PON1 L55M								
LL	43 (26.5)	22 (15.4)	1.86	0.7–4.9	0.2	0.67	0.1–2.5	0.56
LM	32 (19.7)	18 (13.1)	2.8	1.4–18.0	0.02	1.57	0.1–17.3	0.7
MM	29 (17.8)	9 (6.5)	4.33	1.7–10.5	0.001	5.46	1.9–17.0	0.007
PON1 Q192R								
QQ	68 (42)	41 (29.2)	3.5	1.7–7.26	0.001	3.12	1.3–6.9	0.005
QR	29 (18)	6 (4.3)	2.7	0.9–13.7	0.09	2.5	0.6–10.2	0.18
RR	7 (4)	2 (1.5)	1.4	0.11–1.9	0.16	0.6	0.1–18.3	0.8

PON1 activity: 90 IU/L median

[a] Adjusted for age, BMI, lipid profile

to play an important role in lipid metabolism. PON1 significantly decreases lipid peroxidase generation during LDL oxidation in the presence of HDL modification by lipid peroxidase [16, 17]. Smoking impairs PON1 activity and thereby compromises antioxidant defense mechanism [18]. Moreover, a decrease in PON1 activity in smokers can be explained by the effects of several of the hundreds of chemical components of tobacco smoke that have been shown to be responsible for inhibition of PON1 activity, including various reactive aldehydes (acetaldehyde, formaldehyde, and α,β-unsaturated aldehydes such as acrolein and crotonaldehyde) as well as aromatic hydrocarbons [19]. In this respect, several studies showed that smoking was associated with reduced serum PON1 activity [20]. Factors affecting PON1 levels are listed as: dietary and lifestyle factors, alcohol intake, vitamin C and E intake, and the most popular factor, *PON1* gene polymorphism. In addition, urinary cotinine and plasma SCN^- concentrations were both significantly higher in smokers than in nonsmokers. Although urine cotinine and plasma SCN^- are influenced by diet and industrial pollution, they remain reliable indicators of smoking status [21]. We noted significant association between smoking status and lower PON1 activity before and after adjustments for confounder factors. Cigarette smoke has high content of oxidants that promote a prooxidant effect in blood plasma and tissues, which probably contributes to the increased incidence of cardiovascular disease among smokers. The information available on the molecular mechanisms of action of cigarette smoke is limited. However, recent observations suggest that the prooxidant effect of smoking is, in part, related to PON1 activity inhibition caused by cigarette smoke [18]. The results presented here indicate a critical role for amino acid substitutions at both positions 55 and 192 and cigarette smoking in determining the activity of PON1 towards lipid peroxides. Moreover, we found significant variations in serum PON1 activity depending on genetic polymorphisms. PON1 activity increased as a function of genotypes in the order 192RR > 192QR > 192QQ and 55LL > 55LM > 55MM, as has also been described in other populations [22, 23]. The adjusted model confirmed that the PON1 L55M and PON1 Q192R polymorphisms influenced paraoxonase salt-stimulated activity, without significant contribution to enzyme activity of any other predictors considered in this study.

Minimal information is available at the molecular level concerning the mechanism of action of cigarette smoke. Nishio et al. [7] recently demonstrated that a cigarette smoke extract inhibits PON1 activity in a dose- and time-dependent manner. Inhibition of paraoxonase by smoke might be caused by steric hindrance resulting from the introduction of a large substituent near a region of the molecule critical for substrate binding or the maintenance of an active enzyme conformation. However, nothing is known about the effect of PON1 genotypes on inhibition of PON1 activity caused by smoking. PON1 activity decreased in smokers in comparison with nonsmokers. Therefore, both PON1 L55M and PON1 192 polymorphisms and smoking-induced degradation of PON1 may be synergistically involved in reduction of PON1 activity in the airways [24].

Furthermore, levels of HDLc were significantly decreased in smokers compared with nonsmokers regardless of PON1 L55M polymorphisms, confirming the effect of cigarette smoking on decrease of HDLc concentrations.

In smokers, we demonstrated that HDLc levels were significantly decreased in PON1 RR compared with PON1 QQ. Previous studies have investigated the association between PON1 192 polymorphism and blood lipids. HDL may play a significant role in the effect of PON1 on coronary disease. Several lines of evidence have been provided for a potential biological link between the Q/R192 polymorphism and the antiatherogenic effect. Mackness et al. [25] demonstrated that HDL containing R192 PON1 was less effective in protecting LDL from oxidative modification than HDL with Q192 PON1. These findings may explain why the paraoxonase R allele has been found to be present at increased frequency in coronary heart disease,

leading to the hypothesis that PON1 192 polymorphism might be a risk factor for atherosclerosis, although this was not confirmed in other studies.

After adjustment for confounding variables, smokers with PON1 L55M polymorphism had the highest risk for lower PON1 activity; however, PON1 Q192R genotype might protect smokers from decrease in PON1 activity. We found significant interaction between the effect of cigarette smoking and both PON1 L55M and PON1 Q192R polymorphisms on lower PON1 activity.

We found a negative correlation between paraoxonase 1 activity and both urinary cotinine concentration and plasma SCN^- concentration in smokers. The important correlation found between urinary cotinine and plasma uric acid in smokers was not surprising, because urinary cotinine and plasma SCN^- levels were determined as markers of tobacco smoke exposure [21].

In conclusion, cigarette smoking was significantly associated with decrease in PON1 activity. Moreover, PON1 L55M polymorphism predisposes smokers to decrease in PON1 activity in contrast to PON1 Q192R genotype. This finding suggests that these polymorphisms may play a role in development of cardiovascular diseases in smokers.

Acknowledgments The authors thank Mr. Samir Boukattaya for his assistance with English language correction.

Conflict of interest The authors state that there are no conflicts of interest regarding the publication of this article.

References

1. Fakhfakh R, Jendoubi W, Achour N. Le Tabagisme chez les étudiants paramédicaux de Tunis. Tunis Med. 2010;88:534–44.
2. Mahadesh Prasad AJ, Kemparaju K, Elizabeth AF, Jaichander P, Arun A, Cletus JM. Lack of correlation of paraoxonase (PON1) activity with smoking among the south Indians and risk of cardiovascular disease. World Appl Sci J. 2010;9:194–8.
3. Ferre N, Camps J, Fernandez-Ballart J, Arija V, Murphy MM, Ceruelo S. Regulation of serum paraoxonase activity by genetic, nutritional, and lifestyle factors in the general population. Clin Chem. 2003;49:1491–7.
4. Mackness B, Durrington PN, Mackness M. Polymorphisms of paraoxonase genes and low-density lipoprotein peroxidation. Lancet. 1999;353:468–9.
5. Leviev I, James RW. Promoter polymorphisms of the human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. Arterioscler Thromb Vasc Biol. 2000;20:516–21.
6. James RW, Leviev I, Righetti A. Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease. Circulation. 2000;101:2252–7.
7. Nishio E, Watanabe Y. Cigarette smoke extract inhibits plasma paraoxonase activity by modification of the enzyme's free thiols. Biochem Biophys Res Commun. 1997;236:289–93.
8. Toma′s M, Sentı M, Garcı′a-Faria F, Vila J, Torrents A, Covas M, et al. Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. Arterioscler Thromb Vasc Biol. 2000;20:2113–9.
9. Sutherland W, Manning PJ, de Jong SA, Allum AR, Jones SD, Williams SM. Hormone-replacement therapy increases serum paraoxonase arylesterase activity in diabetic postmenopausal women. Metabolism. 2001;50:319–24.
10. López-Flores I, Lacasaana M, Blanco-Munozc J, Aguilar-Garduno C, Sanchez-Villegas P, Pérez-Méndez OA, et al. Relationship between human paraoxonase-1 activity and PON1 polymorphisms in Mexican workers exposed to organophosphate pesticides. Toxicol Lett. 2009;188:84–90.
11. Kallel A, Sediri Y, Sbaï MH, Mourali MS, Feki M, Elasmi M, et al. The paraoxonase L55M and Q192R gene polymorphisms and myocardial infarction in a Tunisian population. Clin Biochem. 2010;43:1461–3.
12. Araoud M, Neffeti F, Douki W, Kenani A, Najjar MF. Development of an automated method for the determination of human paraoxonase 1 activity. Asian Biomed. 2011;5:217–24.
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.
14. Motti C, Dessí M, Gnasso A, Irace C, Indigeno P, Angelucci CB, Bernardini S, Fucci G, Federici G, Cortese C. A multiplex PCR-based DNA assay for the detection of paraoxonase gene cluster polymorphisms. Atherosclerosis. 2001;158:35–40.
15. Meenakshisundaram R, Rajendiran C, Ponniah T. Lipid and lipoprotein profiles among middle aged male smokers: a study from southern India. Tob Induc Dis. 2010;8:11.
16. Li H, Liu D, Liang C. Paraoxonase gene polymorphisms, oxidative stress, and diseases. J Mol Med. 2003;81:766–79.
17. Oda M, Bielicki J, Ho T, Berger T, Rubin E, Forte T. Paraoxonase 1 over expression in mice and its effect on high-density lipoproteins. Biochem Biophys Res Commun. 2002;290:921–7.
18. Haj Mouhamed D, Ezzaher A, Neffati F, Douki W, Najjar MF. Paraoxonase 1 activity and lipid parameters in Tunisian smokers. Ann Biol Clin. 2010;68:143–7.
19. Lucio GC, Annabella V, Toby BC, Clement EF. Modulation of paraoxonase activity. Biochem Pharm. 2005;69:541–50.
20. Solak ZA, Kabaroglu C, Çok G, Parıldar Z, Bayındır Ü, Özmen D, Bayındır O. Effect of different levels of cigarette smoking on lipid peroxidation, glutathione enzymes and paraoxonase 1 activity in healthy people. Clin Exp Med. 2005;5:99–105.
21. Haj Mouhamed D, Ezzaher A, Neffati F, Douki W, Najjar MF. Comparison between plasma and urine thiocyanates and urinary cotinine determinations as indicators of cigarettes smoking. J Health Sci. 2009;55:1–6.
22. Rojas-García AE, Solís-Heredia MJ, Pina-Guzmán B, Vega L, López-Carrillo L, Quintanilla-Vega B. Genetic polymorphisms and activity of PON1 in a Mexican population. Toxicol Appl Pharm. 2005;205:282–9.
23. Catano H, Cueva JL, Cardenas AM, Izaguirre V, Zavaleta A, Carranza E, et al. Distribution of paraoxonase-1 gene polymorphisms and enzyme activity in a Peruvian population. Environ Mol Mutagen. 2006;47:699–706.
24. Seo T, Pahwa P, Mcduffie HH, Nakada N, Goto S, Ghosh S, et al. Interactive effect of paraoxonase-1 Q192R polymorphism and smoking history on the Lung Function decline in grain workers. Ann Epidemiol. 2008;18:330–4.
25. Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. FEBS Lett. 1998;423:57–60.