Turk J Endocrinol Metab 2018;22:151-157

Paraoxonase 1 Activity and its Polymorphism in Type 2 Diabetic Nephropathy

Tip 2 Diyabetik Nefropatide Paraoksonaz 1 Aktivitesi ve Polimorfizmi

Mukund R. Mogarekar, Mahendrakumar G. Dhabe, Mayuri M. Palmate

Department of Biochemistry, S.R.T.R. Govt. Medical College, Ambajogai, Maharshtra, India

Abstract

Objective: This study aims to investigate the role of paraoxonase 1 activity and Q192R polymorphism in the development of nephropathy in patients with Type 2 diabetes mellitus.

Material and Methods: This case-control study included 100 patients with Type 2 diabetes mellitus for more than five years, admitted to our hospital. They were divided into two groups (with and without diabetic nephropathy) on the basis of albumin-creatinine ratio. Serum samples of all patients were subjected to paraoxonase 1 arylesterase activity, using phenylacetate as the substrate. Paraoxonase 1 phenotyping was carried out by calculating the ratio of inhibited arylesterase activity to non-inhibited arylesterase activity using phenylacetate and p-nitrophenyl acetate, respectively, as substrates.

Results: Paraoxonase 1 arylesterase activity was found to be significantly lower in subjects with diabetic nephropathy than that in the subjects without diabetic nephropathy (85.4±24.12 vs. 127.94±25.51 p=0.01). Bothunivariate (Odds ratio=1.073, Neglekerke's R2=0.565, area under the curve=0.888, p≤0.001) and multivariate (Odds ratio= 1.067, [95% confidence interval (1.023-1.113), p=0.003)] logistic tests showed independent, protective association of paraoxonase 1 arylesterase activity with the development of diabetic nephropathy. Paraoxonase 1 RR homozygous diabetic patients were observed to be significantly associated with diabetic nephropathy in the univariate logistic regression (R2=0.056 area under the curve=0.600 p=0.037), while the multivariate analysis did not show any significance.

Conclusion: A decreased paraoxonase 1 arylesterase activity may be considered to be an additional risk factor in the development of nephropathy in diabetes mellitus. Paraoxonase 1 RR homozygous individuals may be at an increased risk of being affected by diabetic nephropathy.

Keywords: Paraoxonase 1; polymorphism; diabetic nephropathy

Özet

Amaç: Bu çalışmada, Tip 2 diabetes mellitusu olan hastalarda nefropati gelişiminde paraoksonaz 1 ve Q192R polimorfizminin etkinliğinin araştırmaktır.

Gereç ve Yöntemler: Bu vaka-kontrol çalışmasına, hastanemize başvuran ve beş yıldan fazla süredir diabetes mellitusu olan 100 hasta alındı. Hastalar, albümin/kreatinin oranına göre iki gruba ayrıldı (diyabetik nefropatisi olan ve olmayan). Tüm hastaların serum örnekleri substrat olarak fenilasetat kullanılarak paraoksonaz 1 arilesteraz aktivitesine tabi tutuldu. Paraoksonaz 1 fenotiplendirmesi, inhibe olan arilesteraz aktivitesinin inhibe olmamış arilesteraz aktivitesine oranının substrat olarak sırasıyla fenilasetat ve p-nitrofenil asetat kullanarak hesaplanmasıyla gerçekleştirildi.

Bulgular: Diyabetik nefropatisi olan hastalarda, diyabetik nefropatisi olmayanlara göre paraoksonaz 1 arilesteraz aktivitesi anlamlı olarak daha düşük bulunmuştur (sırasıyla, 85,4±24,12 ve 127,94±25,51; p=0,01). Hem tek değişkenli (Odds oranı=1.073, Neglekerke's R2=0,565, eğri altında kalan alan=0,888, p≤0.001) hem de çok değişkenli (Odds oranı=1,067, [%95 güven aralığı (1,023-1,113), p=0,003] lojistik testler paraoksonaz 1 arilesteraz aktivitesinin diyabetik nefropatisi gelişimi açısından bağımsız koruyucu ilişkisini ortaya koymuştur. Tek değişkenli analizlerde, paraoksonaz 1 RR homozigot diyabetik hastaların diyabetik nefropatisi ile anlamlı ilişki gösterdiği gözlenmiş (R2=0,056 eğri altında kalan alan=0,600 p=0,037), fakat aynı ilişki çok değişkenli analizlerde gösterilememiştir.

Sonuç: Azalmış paraoksonaz 1 arilesteraz aktivitesi diabetes mellitus hastalarında nefropati gelişimi açısından ek bir risk faktörü olarak kabul edilebilmektedir. Paraoksonaz 1 RR homozigot bireyler diyabetik nefropatisi açısından artmış risk altında olabilmektedir.

Anahtar kelimeler: Paraoksonaz 1; polimorfizm; diyabetik nefropati

Address for Correspondence: Mukund R. Mogarekar, Department of Biochemistry, S.R.T.R. Govt. Medical College, Ambajogai, Maharshtra, India
Phone: +91 9421345546 **E-mail:** mrmogarekar@hotmail.com **Received:** 18/02/2018 **Accepted:** 16/05/2018

DOI: 10.25179/tjem.2018-60184

Introduction

Diabetes mellitus, the most common disease, accounted for around 350 million cases in 2014 (1). The prevalence of diabetes was estimated to be about 8% worldwide in 2011 and was predicted to increase up to 10% by 2030 (2). The chronic complications diabetes include damage, dysfunction and/or failure of various organs especially the retina, kidneys, nerves, heart, and vessels (3). Severe complications of diabetes such as retinopathy, neuropathy, nephropathy and cardiovascular complications are the results of either tissue or vascular damage (4).

DN is a microvascular complication of diabetes. This kidney disease develops in 20–40% of the patients with diabetes (5). The risk factors for the development of DN include hyperglycemia, hypertension, dyslipidemia, smoking and a family history of DN (6). Hyperglycemia occurs first, leading to the development of different pathological events which cause the overt proteinuria, ultimately resulting in glomerulosclerosis and End Stage Renal Disease (7).

It is largely found in circulation in forms bound to HDL. The protective effects of HDL are predominantly due to PON1 (8). PON1 hydrolyzes the phospholipids and hydroperoxides to oxidized LDL and also destroys the proinflammatory molecules involved in the initiation and progression of microvascular complications in diabetes (9). PON1 helps in maintaining the functions of HDL by preventing its oxidation, preserves the antiatherogenic functions of HDL, and reduces the oxidation of LDL (10).

Q and R alleles of polymorphism at position 192 combine to give three phenotypes: QQ, QR, and RR. The Q192R polymorphism markedly influences the enzyme activity for different substrates (11). 192RR individuals show the highest paraoxonase activity, while 192QQ individuals demonstrate a higher lactonase activity (12). Although several studies have demonstrated the relationship between PON1, Q192R polymorphism, and coronary artery disease; yet,other studies focusing on Q192R site could not identify the polymorphism an independent risk factor (13, 14).

The literature reports many studies correlating paraoxonase polymorphism in DM patients. S. Araki et al. observed that there was no association between the PON1 polymorphism and DN (15), while Gokcen et al. observed a significant association between the PON1 polymorphism and DM (16). As different studies present conflicting results there is a need for further research to study the effect of DN on serum PON1 status.

The present study was designed to investigate whether the PON1 arylesterase activity and its Q192R polymorphism are associated with DN in a group of patients with T2DM, besides other risk factors.

Material and Methods

One hundred patients, having diabetes for more than five years, admitted to SRTR Govt. Medical College and Hospital were randomly selected. The patients having DN were compared with those without nephropathy. Age and sexmatched controls were selected from the diabetics who did not have any complication and attended the regular medical check-up in the hospital. Nephropathy was determined by calculating Urinary Albumin/Creatinine Ratio (UACR).

UACR<30 mg/gm: normoalbuminuria (mg of albumin and gm of creatinine)

UACR=30-300 mg/gm: microalbuminuria (mg of albumin and gm of creatinine)

UACR >300 mg/gm: macroalbuminuria (mg of albumin and gm of creatinine).

Patients with UACR >30 mg/gm were selected.

The protocol for this study was approved by the institutional ethics committee of the Medical College. Written informed consent was obtained from all the patients. The patients with associated ischemic heart disease, rheumatoid arthritis, concomitant liver, kidney diseases other than DN, a history of exogenous hormone administration and those having DM for a period of less than five yearswere excluded from the study. Allthe patients included in the study were questioned in detail regarding the disease history.

Biochemical Analysis

All the fine chemicals were obtained from Sigma-Aldrich, Mumbai, Maharashtra, India Venous blood samples were collected aseptically from all patients in the morning, after an overnight fast. Random samples of urine from the same patients were also obtained and were subjected to estimation of urinary albumin and creatinine usingpyro-

gallol red method (17) and Jaffe's alkaline picrate method (18), respectively. The serum was separated by low-speed centrifugation and was subjected to various biochemical analyses. Random blood samples collected in fluoride bulb were analyzed within a few hours for the estimation of plasma glucose using glucose oxidase-peroxidase method (19).

Measurement of PON1 arylesterase activity

Phenylacetate was used as a substrate for determining the arylesterase activity. The rate of hydrolysis was measured spectrophotometrically, using the assay mixture which contained 4.0 mM/L phenylacetate and 1 mM/L $CaCl_2$ in 20 mM/L Tris HCl buffer at a pH 8.0 and temperature 25 °C (20). The rate of phenol formation was recorded at 270 nm following 20 s lag time. The activity was expressed as kU/L, based on the extinction coefficient of phenol, which was noted to be 1310 $M^{-1}cm^{-1}$ at 270 nm, pH 8.0 and 25 °C. Intra assay coefficient of variation (CVs) was 3.2%.

PON1 polymorphism

Each study subject was phenotyped for the PON1 Q192R polymorphism by using the dual substrate method. The ratio of inhibited to the non-inhibited activity which represents the ratio of phenylacetate inhibited arylesterase activity (IA) with p-nitro phenyl acetate as a substrateto non-inhibited arylesterase activity (NIA) with p-nitrophenyl acetate alone as a substrate, was taken into consideration. The rate of formation of p-nitrophenol was determined at 405 nm at a temperature of 25 ºC over 225 s, following 100 slag time. The activity was expressed in kU/L, based on the molar absorptivity (14000 $M^{-1}cm^{-1}$ min^{-1}) of p-nitrophenol at 405 nm and a pH 7.4 (21).

Lipid profile in diabetic nephropathy

The levels of total cholesterol, HDL cholesterol and triglycerides were measured using enzymatic techniques; LDL cholesterol was calculated using the Friedewald formula (22).

Statistical Analysis

The esults presented as mean±standard deviation. The continuous variables were tested for normality using Shapiro-Wilk test. The statistical analysis for the numerical variables in Gaussian distributionwas carried out using Student's unpaired t-test. The number of individuals with each phenotype was compared using the chi-square test. The deviation of observed allele frequency, from those predicted by Hardy-Weinberg equilibrium, was evaluated by the chi-square test. The univariate and multivariate logistic regression analyses were used for assessing the risk of DN, as contributed by various risk factors.

The results were analyzed using the Mystat–12 statistical software. The significance level was assigned to be <0.05.

Results

One hundred patients having diabetes for more than five years and admitted to the SRTR Govt. Medical College and Hospital were selected for the study (50 patients with DN and, 50 type 2 diabetic patients without nephropathy were selected as controls).

The mean age of the individuals included in the study was 56±10.48 and 58.82±9.33 years in the control and patient group, respectively. While no significant difference in age and sex between the patients and controls was observed, Student`s t-test showed that parameters like duration of diabetes, Random BSL, total cholesterol and LDL-C cholesterol were significantly higher in the patient group as compared to the controls. There was no significant difference in TG, VLDL-C, and HDL-C among the patients and controls. PON1 arylesterase activity was found to be significantly lower in subjects as compared to that in the controls (Table 1). The phenotypic distribution of PON1 polymorphism (QQ, QR, and RR phenotypes) was not significantly distinct between the patients and controls (Table 2). PON1 arylesterase activity showed a significant difference in terms of QQ, QR and RR phenotypes between the patients and controls (Table 3).

In the univariate logistic regression, considering DN as a dependent factor, all parameters except serum triglyceride, HDL-C, and VLDL-Showed a significant contribution to the risk of nephropathy in diabetic patients (Table 4). The significant parameters univariate regression were modeled through multivariate regression ands-R and PON1

Table 1. Clinical characteristic and laboratory data in cases and controls.

Parameters	Controls	Subjects	p value
Age (years)	56±10.48	58.82±9.33	0.158
Sex (male/female)	35/15	38/12	0.499
Duration of diabetes	8.54 ±1.35	9.16±1.47	0.035
Random BSL (mg/dL)	151.7±23.40	205.18±41.20	<0.001
Total cholesterol (mg /dL)	150.68±22.99	168.92±23.17	<0.001
Triglyceride (mg/dL)	164.7±26.11	171.26±26.69	0.225
HDL-C	43.86±4.77	42.1±7.27	0.151
VLDL-C	32.94±5.22	34.25±5.54	0.225
LDL-C	73.88±12.99	92.57±10.36	0.001
PON1 arylesterase (KU/L)	127.94±25.51	85.4±24.12	<0.001

HDL-c =High Density Lipoprotein cholesterol; LDL-c= Low Density Lipoprotein cholesterol; PON1= Paraoxonase 1.

Table 2. Distribution of PON1 Phenotype in controls and subjects.

No. of individuals with each phenotype (controls)	No. of individuals with each phenotype (subjects)
QQ=15	QQ=12
QR=23	QR=25
RR=12	RR=13
Total=50	Total=50

PON1 polymorphism.

Table 3. Distribution of PON1 arylesterase activity according to phenotype.

Phenotype	Control	Subjects	p Value
PON1 Arylesterase activity			
QQ	130.8±27.65	105.75±21.37	0.016
QR	124.65±22.07	89.36±17.66	<0.001
RR	117.25±14.71	85.5±30.97	0.003

Table 4. Univariate logistic regression analysis.

Parameters	Odds ratio	Neglekerke's r2	AUC	p
BSL-R	0.937	0.603	0.906	<0.001
Duration of DM	0.731	0.062	0.613	0.029
T. cholesterol	0.966	0.183	0.708	<0.001
Triglyceride	0.991	0.020	0.559	0.220
HDL-C	1.049	0.027	0.559	0.151
VLDL-C	0.955	0.020	0.559	0.220
LDL-C	0.968	0.176	0.705	0.001
Arylesterase	1.073	0.565	0.888	<0.001
RR Phenotype	2.488	0.056	0.600	0.037

HDL-c =High Density Lipoprotein cholesterol; LDL-c= Low Density Lipoprotein cholesterol; PON1= Paraoxonase 1; BSL-R = Random blood sugar level; DM. Diabetes mellitus.

arylesterase activity showed an independent, significant contribution in the nephropathy in diabetes along with other parameters; however, in combination with other known parameters, RR phenotype lost its significance as observed in the multiple logistic regression (Table 5).

Discussion

Genetic predisposition, quality of glycemic-control, duration of diabetes, level of blood pressure, and smoking are some of the known risk factors for DN (23). Besides these, the role of other factors in the development of DN was also seen in the study.
evidently found in thestudy that the duration of diabetes in thepatients was significantly higher than that in the controls (9.16±1.47 VS 8.54±1.35, p=0.035). LDL-C was found to be significantly increased in the patient group (92.57±10.36 VS 73.88 ±12.99 p=0.001) and was also significantly associated with DN, as ascertained by the univariate regression (OR=0.968 R^2=0.001). Similar results were observed by The Pittsburgh Epidemiology of Diabetes Complications Study which identified LDL cholesterol as a predictor of microalbuminuria (24). In the present study, PON1 arylesterase activity in the patient group (85.4 ±24.12 kU/L) was observed to be significantly lower than that in the control group (127.94±25.51 kU/L) (p<0.001). Univariate logistic regression considerably indicated it to bea protective factor in the development of DN, showing an odds ratio of 1.073 and Neglekerke's R^2 value of 0.565 (p<0.01). Multivariate logistic regression, which was also in combination with other variables, showed that PON1 arylesterase activity contributed significantly to the development of nephropathy in diabetes. LI C et al. (2009) (25) and El-said N. H. et al. (2015) (26) also found that patients with nephropathy had a lower PON1 activity as compared to their controls, which is in line with the present study. In DN, PON1 glycation causes dissociation of PON1 from HDL, making PON1 less stable; glycation also impairs the reverse cholesterol transport ability of HDL, all of which may cause a decrease in PON1 activity as evident in this study, which was confirmed by Rosenblat M et al. (27) and Hedrick CC et al. (28). Tabur S. et al. (29) explained that DN is a state of oxidative stress, in which PON1 arylesterase activity is decreased; an antioxidant enzyme has been reported to be associated with an increase in the reactive oxygen radicals and oxidative stress.
In the present study, phenotype-wise distribution of PON1 Q192R polymorphism showed no significant difference between the patients and controls. RR homozygotes were observed to be independently associated with the development of DN in the univariate logistic regression; however, in the multivariate logistic regression, it lost its significance. Q192 provides protection against microangiopathic, probably due to the effective hydrolysis of hydroperoxides and peroxides of PON1 QQ, as established by Murata M et al. (2004) (30) in their study. Hampe M et al. (2014), in a cross-sectional study, investigated the role of paraoxonase 1 (PON1) status such as activity and Q192R polymorphism in the development of Diabetic Retinopathy (DR) in patients with type 2 DM, and found that retinopathy was significantly associated with PON1 R allele carriers in diabetic patients

Table 5. Multivariate logistic regression.

Model (R^2=0.726AUC=0.945 P<0.001)				
BSL-R	-0.039	0.962	0.938-0.982	<0.001
Duration of DM	0.026	1.026	0.620-1.479	0.914
TC	0.036	1.036	0.941-1.133	0.455
LDL	-0.043	0.958	0.878- 1.046	0.345
Arylesterase	0.065	1.067	1.023- 1.113	0.003
RR phenotype	-0.846	0.429	0.071- 2.584	0.356

The dependent factor is the presence or absence of disease (Cases are taken as reference).
LDL= Low Density Lipoprotein; BSL-R = Random blood sugar level; DM: Diabetes mellitus.

(71.05%) than with diabetic patients homozygous for PON1 QQ (29.41%) (31), which is in line with the present study. Inoue M**.** et al. (2000) (32) found that PON1 arylesterase activity was significantly lower in diabetes with nephropathy, but the lower activity was independent of PON1 192Q/R polymorphism. A meta-analysis done by Wang J. et al. in 2013 concluded that PON1 Q192R polymorphism may not be associated with DN and DR (33). The reasons for these discrepancies may be the differences in the type of diabetes, ethnicity, and other genetic contributors. More extensive and preferably prospective studies are required to confirm the results of the present study. The main limitation of this study is the small sample size, which is not enough to establish the predictive value of PON-1 polymorphism in DN. PON1 genotyping, which is more definitive, has not been performed in the present study. Thus, a false classification of few individuals, among the three phenotypes, could have been possible. Further, the actual concentration of PON1 has not been directly calculated in the present study and a larger cohort study is therefore required to serve this purpose.

Conclusion

The authors ultimately conclude according to the results of the present study that the PON1 arylesterase activity in diabetic patients with nephropathy is reduced as compared to that in the diabetic patients without nephropathy, due to an increase in the oxidative stress and PON1 glycation in DN. Diabetic patients having R allele are susceptible to the development of nephropathy in the course of diabetes, thus,PON1 status is an additional risk factor for the development of nephropathy along with the known risk factors already prevailing.

Compliance with Ethical Standards

Ethical approval- All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee. (vide letter no. 258/2015 of Institutional Ethics Committee of S.R.T.R.GOVT Medical College, dated 30.11.2015)

Informed consent- A written Informed consent was obtained from all individual participants included in the study which had been approved by the institutional Ethics Committee of S. R. T. R. Govt. Medical College. Ambajogai.

Source of Finance: During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest: No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Mukund R. Mogarekar; Design: Mayuri M. Palmate; Control/Supervision: Mukund R. Mogarekar; Data Collection and/or Processing: Mayuri M. Palmate; Analysis and/or Interpretation: Mukund R. Mogarekar; Literature Review: Mahendrakumar G. Dhabe; Writing the Article: Mayuri M. Palmate; Critical Review: Mahendrakumar G. Dhabe; Materials: Mayuri M.Palmate.

References

1. International Diabetes Federation. IDF Diabetes Atlas Update Poster, 6th ed.; International Diabetes Federation: Brussels, Belgium, 2014.
2. Akter S, Rahman MM, Abe SK, Sultana P. Prevalence of diabetes and prediabetes and their risk factors among Bangladeshi adults: a nationwide survey. Bull World Health Organ. 2014;92:204-213A.
3. American Diabetes Association. Microvascular complications and foot care diabetic. Diabetes Care. 2017;40:S88-99.
4. Reusch JE. Diabetes, microvascular complications, and cardiovascular complications : what is it about glucose? J Clin Invest. 2003;112:986-988.
5. Tuttle KR, Bakris GL, Bilous RW, Chiang JL, de Boer IH, Goldstein-Fuchs J, Hirsch IB, Kalantar-Zadeh K, Narva AS, Navaneethan SD, Neumiller JJ, Patel UD, Ratner RE, Whaley-Connell AT, Molitch ME. Diabetic kidney disease: a report from an ADA consensus conference. Diabetes Care. 2014;37: 2864-2883.
6. Lewis JB. Harrison's Nephrology and Acid-Base Disorders. (17th ed). In: Jameson JL, Loscalzo J, eds. MC Graw-Hill; 2010;171-172.
7. Vinod PB. Clinical queries: nephrology pathophysiology of diabetic nephropathy. Clin Queries Nephrol. 2012;1:121-126.

8. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol. 2001;21:473-480.
9. Ng CJ, Shih DM, Hama SY, Villa N, Navab M, Reddy ST. The paraoxonase gene family and atherosclerosis. Free Radic Biol Med. 2005;38:153-163.
10. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest. 1998;101:1581-1590.
11. Ruiz J, Blanché H, James RW, Garin MC, Vaisse C, Charpentier G, Cohen N, Morabia A, Passa P, Froguel P. Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. Lancet. 1995;346:869-872.
12. Rainwater DL, Rutherford S, Dyer TD, Rainwater ED, Cole SA, Vandeberg JL, Almasy L, Blangero J, Maccluer JW, Mahaney MC. Determinants of variation in human serum paraoxonase activity. Heredity (Edinb). 2009;102:147-154.
13. Antikainen M, Murtomäki S, Syvänne M, Pahlman R, Tahvanainen E, Jauhiainen M, Frick MH, Ehnholm C. The Gln-Arg191 polymorphism of the human paraoxonase gene (HUMPONA) is not associated with risk of coronary artery disease in Finns. J Clin Invest. 1996;98:883-885.
14. Gardemann A, Philipp M, Hess K, Katz N, Tillmanns H, Haberbosch W. The paraoxonase leu-Met54 and gln-Arg191 gene polymorphisms are not associated with the risk of coronary heart disease. Atherosclerosis. 2000;152:421-431.
15. Akari S, Makita Y, Canani L, Ng D, Warram JH, Krolewski AS. Polymorphism of human paraoxonase 1 gene (PON1) and susceptibility to diabetic nephropathy in type1 diabetes mellitus. Diabetologia. 2000;43:1540-1543.
16. Gokçen S, Cengiz M, Ozaydın A. Serum paraoxonase levels and PON1(192) polymorphism in type 2 diabetes mellitus patients. GMJ. 2013;24:70-73.
17. Watanabe N, Kamei S, Ohkubo A, Yamanaka M, Ohsawa S, Makino K, Tokuda K. Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. Clin Chem. 1986;32:1551-1554.
18. Lamb E. Kidney function tests. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics (5th ed). London: Elsevier: 2012;680-681.
19. Cramp DG. New automated method for measuring glucose by glucose oxidase. J Clin Pathol. 1967;20:910-912.
20. Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet. 1983;35:1126-1138.
21. Mogarekar MR, Chawhan SS. The determination of Q192R polymorphism of paraoxonase 1 by using non-toxic substrate p-nitrophenylacetate. Indian J Hum Genet. 2013;19:71-77.
22. Friedewald WT, Lewy RI, Fredrickson DS. Estimation of concentration of low- density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. Clin Chem. 1972;18:499-502.
23. Ritz E. Nephropathy in type 2 diabetes. J Intern Med. 1999;245:111-126.
24. Jenkins AJ, Lyons TJ, Zheng D, Otvos JD, Lackland DT, McGee D, Garvey WT, Klein RL; DCC/EDIC Research Group. NMR analysis of lipoprotein subclasses in the EDIC cohort: associations with gender and glycemia. Diabetes Care. 2003;26:810-818.
25. Li C, Gu Q. Protective effect of paraoxonase 1 of high-density lipoprotein in type 2 diabetic patients with nephropathy. Nephrology (Carlton). 2009;14:514-520.
26. El-said NH, Nasr-allah MM, Sadik NA, Sharaf SA. Paraoxonase-1 activity in type 2 diabetes mellitus with and without nephropathy. Egypt Soc Intern Med. 2015;27:63-68.
27. Osama MR. Glucose inactivates paraoxonase 1 (pon1) and displaces it from high density lipoprotein (HDL) to a free PON1 form. In: Mackness B, Mackness M, Aviram M, Parag G, eds. The Paraoxonases: Their Role in Disease Development and Xenobiotic Metabolism (6th ed). Netherlands: Springer; 2008;35-49.
28. Hedrick CC, Thrope SR, Fu MX, Harper CM, Yoo J, Kim SM, Wong H, Peters AL. Glycation impairs high-density lipoprotein function. Diabetologia. 2000;43:312-320.
29. Tabur S, Korkmaz H, Eren MA, Oğuz E, Sabuncu T, Aksoy N. Urotensin-II level and its association with oxidative stress in early diabetic nephropathy. J Diabetes Complications. 2015;29:115-119.
30. Murata M, Maruyama T, Suzuki Y, Saruta T, Ikeda Y. Paraoxonase 1 192Gln/Arg polymorphism is associated with the risk of microangiopathy in Type 2 diabetes mellitus. Diabet Med. 2004;21:837-844.
31. Hampe MH, Mogarekar MR. Paraoxonase1, its Q192R polymorphism and HDL-cholesterol in relation to intensive cardiac care unit stay in ischemic heart disease. Indian J Hum Genet. 2014;20:51-58.
32. Inoue M, Suehiro T, Nakamura T, Ikeda Y, Kumon Y, Hashimoto K. Serum arylesterase/diazoxonase activity and genetic polymorphisms in patients with type 2 diabetes. Metabolism. 2000;49:1400-1405.
33. Wang J, Yang MM, Rong SS, Ng TK, Li YB, Liu XM. Association of paraoxonase gene polymorphisms with diabetic nephropathy and retinopathy. Mol Med Rep. 2013;8:1845-1851.