

Genetic polymorphisms in paraoxonase 1 and G protein-coupled receptor 77, and the risk of glucose-6-phosphate dehydrogenase deficiency in a Saudi population

Khalid K. Alharbi, MSc, PhD.

ABSTRACT

الأهداف: دراسة دور الأشكال المختلفة لبدائل الأحماض الأمينية Q192R و C698T في تعويض نقص نازعة الهيدروجين 6 الفوسفاتي الجلوكوزي لدى مجموعة من المرضى السعوديين الذكور.

الطريقة: أُجريت هذه الدراسة المكونة من مجموعتي الشاهد والدراسة في منطقة الطائف، المملكة العربية السعودية وذلك خلال الفترة من يوليو إلى أغسطس 2011م. شملت الدراسة 200 ذكر سعودي، وقد قمنا بتقسيمهم إلى 100 مريض مصاب بنقص G6PD (مجموعة الدراسة)، و100 من الأشخاص الأصحاء (مجموعة الشاهد). لقد قمنا باختيار 100 مريض مصاب بنقص G6PD وذلك من أصل 2100 شخص تم تحليل نتائجهم باستخدام اختبار تبقيع الفلورسين. وبعدها قمنا باستخدام تفاعل البوليمير المتسلسل RFLP وذلك من أجل دراسة النمط الجيني لكلا من PON1 (rs662) و C5L2 (rs149572881).

النتائج: أشارت نتائج الدراسة إلى وجود علاقة بين أليل R والنمط الجيني QR من جهة والتعدد الشكلي للحمض Q192R في النمط الجيني PON1 (R versus Q: OR, 1.72; 95% CI, 1.1-2.6; $p=0.01$, QR versus QQ: OR, 1.98; 95% CI, 1.1-3.6; $p=0.02$) ولقد كان النمط الجيني للحمض C698T وتكرار ظهور الأليل في النمط الجيني C5L2 متشابهة كثيراً بين مجموعتي الشاهد والدراسة (CT versus CC: OR, 2.04; 95% CI, 0.3-11.4; $p=0.40$; and T versus C: OR, 2.02; 95% CI, 0.3-11.1; $p=0.41$).

الخلاصة: تقترح نتائج الدراسة بأنه يمكن أن يكون هنالك علاقة بين PON1 ونقص G6PD لدى المرضى السعوديين الذكور. ونحن بحاجة إلى المزيد من الدراسات من أجل دراسة العلاقة بين الصفات السريرية والنمط الجيني لمختلف الأعراق وتأكيد العلاقة بين الطفرات الجينية وهذا المرض.

Objectives: To investigate the role of amino acid substitution variants Q192R and C698T in the development of glucose-6-phosphate dehydrogenase (G6PD) deficiency in a Saudi male population.

Methods: This case-control study was carried out in 200 Saudi male individuals: 100 patients with G6PD deficiency, and 100 control subjects collected between July and August 2011 in the Taif region of Saudi Arabia. A total of 2100 male Saudi individuals were screened by a fluorescence spot test, and 100 with G6PD deficiency were selected. Two common variants PON1 (rs662) and C5L2 (rs149572881) were genotyped using polymerase chain reaction followed by restriction fragment length polymorphism analysis.

Results: The results showed that the R allele and QR genotype were associated with the Q192R polymorphism in PON1 (R versus Q odds ratio [OR], 1.72; 95% confidence interval [95% CI], 1.1-2.6; $p=0.01$; and QR versus QQ: OR, 1.98; 95% CI, 1.1-3.6; $p=0.02$). All the C698T genotypes and allele frequencies in C5L2 were almost similar in both the cases and controls (CT versus CC: OR, 2.04; 95% CI, 0.3-11.4; $p=0.40$; and T versus C: OR, 2.02; 95% CI, 0.3-11.1; $p=0.41$).

Conclusions: These findings suggest the association of PON1 with G6PD deficiency in the Saudi male population studied herein. Future studies, including correlation analyses between the clinical features and genotypes in populations of different ethnicities, are warranted to confirm the disease association with these genetic mutations.

*Saudi Med J 2015; Vol. 36 (5): 544-548
doi: 10.15537/smj.2015.5.11860*

From the Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia.

Received 25th September 2014. Accepted 26th January 2015.

Address correspondence and reprint request to: Dr. Khalid K. Alharbi, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, PO Box 10219, Riyadh 11433, Kingdom of Saudi Arabia. E-mail: kharbi@ksu.edu.sa

The World Health Organization has confirmed glucose-6-phosphate dehydrogenase (G6PD; E.C.1.1.1.49), an x-linked genetic disorder, to be a public health concern.¹ Glucose-6-phosphate dehydrogenase is an enzyme that converts glucose-6-phosphate into glucose-6-phosphogluconolactone. This conversion is the initial step for the pentose phosphate pathway, and G6PD plays an important role in protecting cells from oxidative damage.²⁻⁴ The G6PD is located at the tip of chromosome Xq28, with 13 exons and 12 introns, and it encodes a 515-amino acid protein. Its deficiency affects nearly 400 million people worldwide.^{5,6} The most common genetic variants in G6PD deficiencies are Mediterranean and A variants, which have been reported in Saudi Arabia.^{4,7,8} Specific genetic polymorphisms associated with G6PD abnormalities have been studied in male subjects in the past.^{4,6,9} No genetic studies on paraoxonase 1 (PON1) or G-protein coupled receptor (C5L2) have been performed in relation to G6PD deficiency. However, these genes have been shown to be associated with a Saudi population.¹⁰⁻¹² This was the key motive for the selection of Q192R and C698T mutation/polymorphisms in G6PD deficiency in this study. Therefore, this study aimed to assess the genotypes of Q192R and C698T, previously associated with genetic, metabolic, and hereditary defects, in Saudi male subjects with G6PD deficiency.

Methods. Ethics statement. The Ethics Committee of the Ministry of Health, Taif City, Saudi Arabia, approved this study. Informed consent was obtained from all the subjects included in the study.

Study population samples. In this study, 2100 male subjects were screened for G6PD deficiency, and 100 (4.7%) were found to be G6PD deficient. Two hundred Saudi male subjects, including 100 individuals with G6PD and 100 normal controls, were enrolled into the present study. Blood samples (6 mL) were obtained from healthy control subjects from the central blood bank, Dallah Driving School, and King Abdulaziz Hospital from the Taif region. All the samples (n=200) were collected between July and August 2011, in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes, and 4 mL of the sample was used for

hematological tests to confirm G6PD deficiency using a fluorescence spot test (Boehringer Mannheim GmGH, Ingelheim am Rhein, Germany). Blood samples with EDTA (2 mL) were used for the molecular analysis. The only inclusion criterion for G6PD deficiency patients was male gender; therefore, female G6PD deficiency patients were excluded based on a questionnaire.⁴

Confirmation of G6PD deficiency. The fluorescence spot test was performed to identify G6PD deficiencies by the reduction of nicotinamide adenine dinucleotide phosphate (NADP) to reduced nicotinamide adenine dinucleotide phosphate (NADPH). This reaction is coupled with the oxidation of glucose-6-phosphate to 6-phosphogluconate, catalyzed by G6PD. Lack of fluorescence under ultraviolet light is considered positive.

DNA extraction. Peripheral blood samples were used for the separation of genomic DNA by Norgen DNA extraction kits (Norgen Biotek Corp, Thorold, ON, Canada). The separated DNA was dissolved in Tris EDTA buffer (final DNA concentration, ~100 ng/μL) and stored at -80°C until further processing. Genotype analysis was performed at the Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia. The procedure followed the standard protocol for DNA extraction.

Genotyping. Polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis was performed. The PCR was carried out to determine the Q192R and C698T variants in PON1 and C5L2. The selection of PCR primers and enzymes for RFLP was based on earlier studies.¹⁰⁻¹² Primers were synthesized by Bio-serve Technologies, Hyderabad, India. The PCR thermal cycling was as follows: DNA denaturation at 95°C for 5 minutes (min); amplification by 35 cycles of 95°C for 30 seconds (s), 60°C (Q192R and C698T) for 30 s, and 72°C for 45 s; and final extension at 72°C for 5 min. The 20-μL reaction mixture contained 2 μL of each primer (10 pmol), 6 μL of sterile water, and 10 μL of 2× master mix (including Magnesium chloride, 10× Taq buffer, 10 U of Taq DNA polymerase; Norgen Biotek Corp, Thorold, ON, Canada), and 2 μL of template DNA. The PCR products were digested at 37°C (2.5 μL of distilled water with 10 U of enzyme for 15 μL of PCR product and 2 μL of buffer in a final volume of 20 μL) and electrophoresed in 2.5% agarose gel containing ethidium bromide.

Statistical analysis. Statistical analysis was performed Open EPI6 software (Open Epi Version 2.3.1 from Department of Epidemiology, Rollins School of Public

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company. This study was funded by the Deanship of Scientific Research Centre, King Saud University, Riyadh, Saudi Arabia (Research Group Project No-RGP -VPP-244).

Health, Emory University, Atlanta, GA 30322, USA). Mean \pm SD of the clinical data of all the subjects (namely, those with and without G6PD deficiency) was determined. Frequencies of both the alleles and genotypes were calculated by the gene counting method. Unpaired data were analyzed by Student's *t*-test. Statistical significance was examined by 2-sided tests. Odds ratio (OR), 95% confidence intervals (CI), and *p*-values were also calculated for the samples. A *p*-value of <0.05 was considered to be statistically significant, and Yates correction was applied for genotyping.

Results. Participants' characteristics. Clinical characteristics of the G6PD deficiency patients and control subjects are summarized in Table 1. The G6PD

deficiency patients were 17-50 years old, whereas in the control group, the age range was 16-52 years ($p=0.87$). The G6PD deficiency was confirmed by the fluorescence spot test. The hematological test yielded statistically significant differences between cases and controls ($p=0.03$). The remaining tests were carried out only in patients and not in the control individuals.

Genotype frequencies for 192Q>T. The PON1 Q192R polymorphism was successfully genotyped in 100 G6PD deficiency patients and 100 control subjects. There was a significant difference in the genotypic distribution and allelic frequencies between patients and control subjects (QR versus QQ: $\chi^2=5.2$; OR 1.98; 95% CI: 1.1-3.6; $p=0.02$). Subjects with G6PD deficiency deviated significantly from the control group for R allele frequency (R versus Q: $\chi^2=6.1$; OR 1.72; 95% CI: 1.1-2.6; $p=0.01$). The genotype and allele frequencies of the Q192R polymorphism between patients and control subjects are shown in Table 2.

Distribution of 698C>T in G6PD deficiency patients and controls. Among the G6PD deficiency patients, 96% showed the CC genotype, and 4% showed the CT genotype. Within the latter genotype, 2% showed the T allele, and 98% showed the C allele. In the control group, 98% showed the CC genotype, and 2% showed the CT genotype. The TT genotype was absent in the entire study population. In the control group, 1% harbored the T allele, while 99% had the C allele. A χ^2 test for the T allele did not show a significance difference between the cases and controls (CT versus CC: OR 2.04; 95% CI: 0.3-11.4; $p=0.40$). None of the genotypes or alleles showed any statistical

Table 1 - Individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency and blood group distribution.

Blood group	G6PD (n=100)	Controls (n=100)	P-value
Age	32.43 \pm 10.05	32.07 \pm 9.89	0.87
Hematology test	10.27 \pm 12.35	7.80 \pm 10.03	0.03
WBCs x10 ³ /uL	3.36 \pm 2.5	NA	NA
RBCs x10 ⁶ /uL	4.84 \pm 0.7	NA	NA
Hb g/dL	13.98 \pm 1.8	NA	NA
Hct %	44.21 \pm 5.7	NA	NA
MCV fl	92.14 \pm 7.8	NA	NA
MCH pg	29.15 \pm 2.5	NA	NA
MCHC g/dL	31.66 \pm 1.4	NA	NA
Plt x10 ³ /uL	242.89 \pm 69	NA	NA

NA - not analyzed/not applicable, WBCs - white blood cells, RBCs - red blood cells, Hb - hemoglobin, HCT - hematocrit, MCV - mean corpuscular volume, MCH - mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, Plt - platelet count

Table 2 - Allele and genotype frequencies for Q192R and C698T variants in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Allele and genotype frequencies	Cases (n=100)	Control (n=100)	χ^2	P-value	Odds ratio	95% confidence intervals
Q192R						
QQ	39 (39.0)	57 (57.0)	Reference	Reference	Reference	
QR	49 (49.0)	36 (36.0)	5.2	0.02	1.98	1.1-3.6
RR	12 (12.0)	7 (7.0)	3.2	0.07	2.50	0.9-6.9
Q	127 (63.5)	150 (75.0)	Reference	Reference	Reference	
R	73 (36.5)	50 (25.0)	6.1	0.01	1.72	1.1-2.6
C698T						
CC	96 (96.0)	98 (98.0)	Reference	Reference	Reference	
CT	4 (4.0)	2 (2.0)	0.68	0.40	2.04	0.36-11.41
TT	0 (0)	0 (0)	0*	0.99*	1	0.01-50.88*
C	196 (98.0)	198 (99.0)	Reference	Reference	Reference	
T	4 (2.0)	2 (1.0)	0.67	0.41	2.02	0.3-11.2

*indicates Yates correction

association (T versus C: OR 2.02; 95% CI: 0.3-11.1; $p=0.41$). Yates correction also failed to yield a positive association in C698T variants (TT versus CC: OR 1.00; 95% CI: 0.01-50.88; $p=0.99$). Table 2 presents the genotype and allele frequencies of C5L2 (C698T).

Blood groups. In this study, 54% of the G6PD deficiency patients had the O positive blood group, followed by 26% with A positive, 10% B positive, and only 1% AB positive. The O and A negative blood groups comprised 3%, B negative 2%, and AB negative 1%.

Discussion. Red cell enzymopathy is one of the most common G6PD deficiencies in humans, estimated to affect 400 million people worldwide.¹³ In total, 160 mutations are responsible for G6PD deficiency, and nearly 10% of the variants at the biochemical and biophysical level has been studied intensively.¹⁴ The geographical distribution of G6PD deficiencies mainly includes the tropical and subtropical regions of the globe, and the frequencies vary throughout these regions. The frequency is high in Africa, Asia, the Middle East, and Mediterranean regions.¹⁵ This study reports PON1 and C5L2 variants in the Saudi population by a PCR-RFLP-based analysis. This study included a small population of G6PD deficiency patients, but Q192R and C698T were not observed as important factors for the early development of G6PD deficiency. In a study by Pinna et al,¹⁵ no positive association was found with gene variants and type 2 diabetes mellitus (T2DM) subjects with retinal complications and G6PD deficiency. Few G6PD studies linked to diabetes and related complications are available in the literature.¹⁵⁻¹⁷ Diabetes might arise due to reduced glutathione, with a low supply of NADPH, thereby elevating the levels of oxidant stress. In this study, we selected male G6PD deficiency subjects because the phenotype in genetic mutations is more likely to be detected in males. Female heterozygotes display variable levels of enzyme deficiency due to the appearance of one of the inactive X chromosomes (Lyonisation). Furthermore, homozygous female subjects with G6PD deficiency are very rare because of the relative improbability of such inheritance.¹⁸ Oxidative stress is known to contribute to cancer development and various disorders, and PON plays an important role in decreasing oxidative stress.¹⁹ The literature suggests that decreased G6PD activity and depletion of blood glutathione leads to an increase in the generation of reactive oxygen species and associated oxidative stress in blood, liver, and brain.¹⁹ The present study was carried out with PON1 (Q192R)

and C5L2 (C698T) variants to ascertain if they play a role in G6PD deficiency in Saudi male individuals. In Saudi populations, the PON1 polymorphism Q192R has been found to be associated with different diseases, including T2DM (unpublished data), gestational diabetes, and coronary artery disease (CAD).¹⁰⁻¹² The current study analyzed PON1 to determine the impact on G6PD deficiency patients vis-a-vis control individuals, and the QR genotype and R allele were found to have a significant association with G6PD deficiency. Ergun et al²⁰ showed a positive correlation of codons 55 and 192 in PON1 with complications of diabetes in a Turkish population. The Q192R polymorphism was associated with CAD, gestational diabetes, obesity, and T2DM plus CAD; however, there was no association with stroke, Alzheimer's disease, or breast cancer.^{10,11,21-25} The mutation C698T in C5L2 has been found to have a positive association with T2DM in a Saudi population.¹² In the year 2011, 2 studies were carried out with the thrombophilic genes MTHFR, FVL, and PII in relation with T2DM in Russian and Iranian populations.²⁶⁻²⁸

On the basis of several earlier studies, this case-control study was carried out in Saudi male individuals affected by G6PD deficiency. The PCR-based assay followed by RFLP or DNA sequencing analysis permitted precise diagnosis for G6PD deficiency for molecular or genetic studies. Genetic polymorphisms provide a robust method for gene mapping and linkage analysis through the human genome project. Most of the mutations in G6PD deficiencies have been identified by PCR-based assays.²⁹

Study limitations. First, the sample size was small ($n=100$) for both cases and controls. Second, there was a lack of female subjects. Third, only one single nucleotide polymorphism was selected from each gene. The final limitation of this study was the failure to measure the hematological data in the control subjects, apart from the fluorescence test. Based on my findings and limitations, we suggest that genotyping should be performed in consanguineous couples affected by G6PD deficiency, particularly in affected homozygous women.

In conclusion, PON1 was found to be associated with G6PD deficiency in a Saudi male population. It is highly recommended that in future, a large number of studies across different ethnicities be conducted to confirm the disease association. Furthermore, correlation between the clinical and genotype analysis should be ascertained.

Acknowledgment. The author would like to extend his sincere appreciation to Mr. Alaa S. Abed and Dr. Imran A. Khan.

References

1. Renzaho AM, Husser E, Polonsky M. Should blood donors be routinely screened for glucose-6-phosphate dehydrogenase deficiency? A systematic review of clinical studies focusing on patients transfused with glucose-6-phosphate dehydrogenase-deficient red cells. *Transfus Med Rev* 2014; 28: 7-17.
2. Olusanya BO, Emokpae AA, Zamora TG, Slusher TM. Addressing the burden of neonatal hyperbilirubinaemia in countries with significant glucose-6-phosphate dehydrogenase deficiency. *Acta Paediatr* 2014; [Epub ahead of print].
3. Relling MV, McDonagh EM, Chang T, Caudle KE, McLeod HL, Haidar CE, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for rasburicase therapy in the context of G6PD deficiency genotype. *Clin Pharmacol Ther* 2014; 96: 169-174.
4. Alharbi KK, Khan IA. Prevalence of glucose-6-phosphate dehydrogenase deficiency and the role of the A- variant in a Saudi population. *J Int Med Res* 2014; 42: 1161-1167.
5. Francis RO, Jhang JS, Pham HP, Hod EA, Zimring JC, Spitalnik SL. Glucose-6-phosphate dehydrogenase deficiency in transfusion medicine: the unknown risks. *Vox Sang* 2013; 105: 271-282.
6. Alharbi KK, Khan IA, Abed AS, Syed R. Insertion/Deletion polymorphisms do play any role in G6PD deficiency individuals in the Kingdom of the Saudi Arabia. *Bioinformation* 2013; 9: 49-53.
7. Gari MA, Chaudhary AG, Al-Qahtani MH, Abuzenadah AM, Waseem A, Banni H, et al. Frequency of Mediterranean mutation among a group of Saudi G6PD patients in Western region-Jeddah. *Int J Lab Hematol* 2010; 32: 17-21.
8. Al-Jaouni SK, Jarullah J, Azhar E, Moradkhani K. Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in Jeddah, Kingdom of Saudi Arabia. *BMC Res Notes* 2011; 4: 436.
9. Alharbi KK, Khan IA, Syed R. Thrombophilic gene polymorphism studies in G6PD deficient individuals from Saudi population. *Bioinformation* 2012; 8: 1255-1259.
10. Hassan MA, Al-Attas OS, Hussain T, Al-Daghri NM, Alokail MS, Mohammed AK, et al. The Q192R polymorphism of the paraoxonase 1 gene is a risk factor for coronary artery disease in Saudi subjects. *Mol Cell Biochem* 2013; 380: 121-128.
11. Al-Hakeem MM, Abotalib Z, Alharbi KK, Khan IA. Relationship between the paraoxonase 1 gene glutamine 192 to arginine polymorphism and gestational diabetes mellitus in Saudi women. *Clin Biochem* 2014; 47: 122-125.
12. Alharbi KK, Khan IA, Syed R. Circulating C5L2 gene polymorphism is associated with type 2 diabetes mellitus in Saudi population. *Mol Biol Rep* 2013; 40: 6323-6327.
13. Phompradit P, Kuesap J, Chaijaroenkul W, Rueangweerayut R, Hongkaew Y, Yamnuan R, et al. Prevalence and distribution of glucose-6-phosphate dehydrogenase (G6PD) variants in Thai and Burmese populations in malaria endemic areas of Thailand. *Malar J* 2011; 10: 368.
14. Gomez-Manzo S, Terron-Hernandez J, de la Mora-de la Mora I, Garcia-Torres I, Lopez-Velazquez G, Reyes-Vivas H, et al. Cloning, expression, purification and characterization of his-tagged human glucose-6-phosphate dehydrogenase: a simplified method for protein yield. *Protein J* 2013; 32: 585-592.
15. Pinna A, Contini EL, Carru C, Solinas G. Glucose-6-phosphate dehydrogenase deficiency and diabetes mellitus with severe retinal complications in a Sardinian population, Italy. *Int J Med Sci* 2013; 10: 1907-1913.
16. Heymann AD, Cohen Y, Chodick G. Glucose-6-phosphate dehydrogenase deficiency and type 2 diabetes. *Diabetes Care* 2012; 35: e58.
17. Cappai G, Songini M, Doria A, Cavallerano JD, Lorenzi M. Increased prevalence of proliferative retinopathy in patients with type 1 diabetes who are deficient in glucose-6-phosphate dehydrogenase. *Diabetologia* 2011; 54: 1539-1542.
18. von Seidlein L, Auburn S, Espino F, Shanks D, Cheng Q, McCarthy J, et al. Review of key knowledge gaps in glucose-6-phosphate dehydrogenase deficiency detection with regard to the safe clinical deployment of 8-aminoquinoline treatment regimens: a workshop report. *Malar J* 2013; 12: 112.
19. Hussein YM, Gharib AF, Etewa RL, ElSawy WH. Association of L55M and Q192R polymorphisms in paraoxonase 1 (PON1) gene with breast cancer risk and their clinical significance. *Mol Cell Biochem* 2011; 351: 117-123.
20. Ergun MA, Yurtcu E, Demirci H, Ilhan MN, Barkar V, Yetkin I, et al. PON1 55 and 192 gene polymorphisms in type 2 diabetes mellitus patients in a Turkish population. *Biochem Genet* 2011; 49: 1-8.
21. Ruperez AI, Lopez-Guarnido O, Gil F, Olza J, Gil-Campos M, Leis R, et al. Paraoxonase 1 activities and genetic variation in childhood obesity. *Br J Nutr* 2013; 110: 1639-1647.
22. Bhaskar S, Ganesan M, Chandak GR, Mani R, Idris MM, Khaja N, et al. Association of PON1 and APOA5 gene polymorphisms in a cohort of Indian patients having coronary artery disease with and without type 2 diabetes. *Genet Test Mol Biomarkers* 2011; 15: 507-512.
23. Zhang G, Li W, Li Z, Lv H, Ren Y, Ma R, et al. Association between paraoxonase gene and stroke in the Han Chinese population. *BMC Med Genet* 2013; 14: 16.
24. Pi Y, Zhang L, Chang K, Li B, Guo L, Fang C, et al. Lack of an association between Paraoxonase 1 gene polymorphisms (Q192R, L55M) and Alzheimer's disease: a meta-analysis. *Neurosci Lett* 2012; 523: 174-179.
25. Saadat M. Paraoxonase 1 genetic polymorphisms and susceptibility to breast cancer: a meta-analysis. *Cancer Epidemiol* 2012; 36: e101-e103.
26. Sibireva OF. Association of hemostatic disorders in diabetic patients with methylenetetrahydrofolate reductase (C677T) and coagulation factors II (G20210A) and V (G1691A) gene polymorphism. *Klin Lab Diagn* 2011; 3: 36-39.
27. Rahimi Z, Felehgari V, Rahimi M, Mozafari H, Yari K, Vaisi-Raygani A, et al. The frequency of factor V Leiden mutation, ACE gene polymorphism, serum ACE activity and response to ACE inhibitor and angiotensin II receptor antagonist drugs in Iranian type II diabetic patients with microalbuminuria. *Mol Biol Rep* 2011; 38: 2117-2123.
28. Rahimi Z, Hasanvand A, Felehgari V. Interaction of MTHFR 1298C with ACE D allele augments the risk of diabetic nephropathy in Western Iran. *DNA Cell Biol* 2012; 31: 553-559.
29. Flora SJ, Mittal M, Pachauri V, Dwivedi N. A possible mechanism for combined arsenic and fluoride induced cellular and DNA damage in mice. *Metallomics* 2012; 4: 78-90.