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Evaluation of the Role of Paraoxonase1 Q192R Polymorphism in Coronary Artery Disease

Koroner Arter Hastalığında Paraoksonaz1 Q192R Polimorfizminin Rolünün Değerlendirilmesi

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ABSTRACT Objetcive: Gene factors play an important role in coronary artery disease (CAD). Atherosclerosis is the most common process of developing CAD. Low density lipoprotein (LDL) oxidation has an essential role in the process of atherosclerosis. The paraoxonase 1 (PON1) enzyme that presents in the HDL structure inhibits LDL oxidation. A number of studies have been conducted around the world, investigating the relation between PON1 gene polymorphism and CAD. Different results have been obtained in various populations; therefore, these findings cannot be generalized to the Iranian population. Consequently, a comprehensive study of the prevalence of PON1 Q192R polymorphism is required. **Material and Methods:** In the present investigation, the study population consisted of 150 subjects, including 102 CAD patients and 48 healthy controls. The Q192R polymorphism of paraoxonase 1 was evaluated in general and also by ethnicity, employing the PCR-RFLP method. **Results:** In general, there was no significant difference ($p>0.05$) in the distribution of QQ, QR, and RR genotypes between patients and controls. In the study by ethnicity, in the Arab ethnic group, in patients with CAD, frequency of QQ genotype was significantly higher, while RR genotype was considerably lower than controls ($p<0.05$). Therefore, QQ genotype could be considered as a risk factor and RR genotype as a protective factor against CAD in the Arab race. **Conclusion:** The Q192R polymorphism of paraoxonase 1 in Arab ethnic group had a significant correlation with CAD. However, no overall association was found between this polymorphism and CAD.

Keywords: Q192R polymorphism of paraoxonase 1; coronary artery disease; genetic difference

ÖZET Amaç: Gen faktörleri koroner arter hastalığında (KAH) önemli rol oynar. Ateroskleroz KAH gelişiminin en yaygın sürecidir. Düşük yoğunluklu protein (LDL) oksidasyonunun ateroskleroz sürecinde önemli rolü vardır. Yüksek yoğunluklu protein (HDL) yapısında yer alan paraoksonaz 1 (PON1) enzimi LDL oksidasyonunu inhibe eder. Dünyada PON1 gen polimorfizmi ile KAH arasındaki ilişkiyi inceleyen çok sayıda çalışma yapılmıştır. Çeşitli populasyonlarda farklı sonuçlar elde edilmiştir; bu nedenle bu bulgular İran populasyonuna genellenemez. Sonuç olarak, PON1 Q192R polimorfizminin prevalansı ile ilgili kapsamlı bir çalışma yapılması gereklidir. **Gereç ve Yöntemler:** Bu araştırmada, çalışma populasyonu 150 olguyu içeriyordu (KAH olan 102 hasta ve 48 sağlıklı kontrol). Paraoksonazın Q192R polimorfizmi PCR-RFLP yöntemi kullanılarak genelde ve etnisiteye göre değerlendirildi. **Bulgular:** Genelde, hastalarla kontroller arasında QQ, QR ve RR genotiplerinin dağılımı açısından anlamlı fark yoktu ($p>0.05$). Etnisiteye göre ise Arap etnik grubunda KAH olanlarda QQ genotpinin sıklığı anlamlı olarak daha yüksekti, RR genotipi konrollere göre anlamlı olarak daha düşüktü ($p<0.05$). Bu nedenle, Arap toplumunda QQ genotipi bir risk faktörü olarak düşünülebilir ve RR genotipi koruyucu bir faktör olarak düşünülebilir. **Sonuç:** Arap etnik grubunda paraoksonaz 1'in Q192R polimorfizmi KAH ile anlamlı olarak korele idi. Fakat bu polimorfizm ile KAH arasında genel bir ilişki bulunmadı.

Anahtar Kelimeler: Q192R polimorfizmi; koroner arter hastalığı; genetik farklılık

Coronary artery disease [CAD] is the leading cause of mortality and morbidity in the world.[1] Risk factor modification would be helpful in reducing mortality and morbidity of CAD.[2] Beside the environmental determinants, genetic factors play an important role in developing CAD.[3] Atherosclerosis is the most essential process in developing CAD.[4] In the process of atherosclerosis, oxidized low density lipoprotein (LDL) leads to formation of foamy cells. These cells play an essential role in formation of fatty streak that

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is the early stage of atherosclerosis. Moreover, oxidized LDL induces proliferation of smooth muscle cells, and platelet aggregation increases apoptosis of macrophages and consequently leads to rupture of the atherosclerotic plaque.[5] High density lipoprotein (HDL) inhibits LDL oxidation in direct and indirect manners by numerous enzymes. It has antioxidative, anti-inflammatory, anti-thrombotic, and anti-apoptotic properties.[6,7] Paraoxonase 1 (PON1) is one of the enzymes in the structure of HDL.This enzyme is a glycosylated protein, containing 354 amino acids and a molecular mass of 47-43 kDa. PON1 increases the binding of HDL to macrophages mediated by abca1, leading to cholesterol efflux from macrophages.[8] PON1 is a member of paraoxonase gene family located on the long arm of chromosome 7 and includes PON1, PON2, PON3 genes.[9] However, it is not yet clear that the change in the function and level of the enzyme has a causal relationship to coronary artery disease or is merely a companion.[10] Results of the studies on the association between the PON1 polymorphisms and CAD differ in various populations. Polymorphisms of the paraoxonase gene associated with CAD include L55M {Leucine to methionine substitution (L/M) at codon 55} affecting the serum PON1 level and Q192R {Glutamine (Q) to arginine (R) substitution (Q/R) at codon 192}, which affects the activity of this enzyme.[11] The results of this study can be used for the following:

1. Screening first-degree relatives of the patients having genotypes predisposing them to CAD.

2. Effective control and modification of risk factors in subjects with genotypes predisposing them to CAD to reduce burden of the disease.

Clinical and paraclinical evaluation of CAD in these subjects for detection of the patient at an earlier stage reduces major cardiovascular events as a result of early diagnosis.

MATERIAL AND METHODS

The study population included 150 subjects, among whom 102 were CAD patients, and 48 were healthy controls. Inclusion criteria for the CAD group were coronary stenosis (more than 50%) in at least one coronary arteries, confirmed by angiography. The control group consisted of healthy subjects with normal coronary artery, confirmed by angiography. We used convenience sampling in our study. All the subjects have been referred to Golestan and Imam Khomeini angiography centers in the year 2017. The study was approved by the ethics committee of the University of Medical Science (ethics code: Ir.ajums.rec.1396.1070). After explaining to the individuals and asking them for participation, questionnaires containing information on ethnicity, age, diabetes, and other CAD risk factors were filled. Then a blood sample of 5 cc was taken from each subject, after angiography, and also in the operating room. The samples were placed in special tubes containing EDTA and stored at -20°C. The statistical analysis obtained using Statistical Package for Social Sciences (SPSS) software. Chi square and ANOVA tests were used to the comparison between two or multiple groups. The p values of less than 0.05 were considered as significant.

DNA EXTRACTION METHOD

200 µl binding buffer and 40 µl of K proteinase was added to 200 µl of blood and mixed by shaking then was incubated at 70° for 10 minutes. After that, 100 µl of pure isopropanol was added and mixed for 5 minutes. Then centrifuged at 8000 x g for several times, using inhibitor removal buffer and wash buffer. And 200 µl of heated elution buffer was added and shaked for 10 minutes. Then, centrifuged for 1 minute at 8,000 x g. PCR reactions were performed using 2.5 µl buffer, 0.7 µl magnesium chloride, 0.5 µl dNTP, 0.5 µl Taq, 1 µl Q192R-F, 1 µl Q192R-R. After that, 5 µl of the genome and finally one drop of mineral oil was added and mixed for a few seconds, the mixture centrifuged for 10 seconds and placed in a thermocycler unit.

RESULTS

Patients were older than controls ($p<0.05$). The mean age in the CAD group was 61.1, and in the healthy control group was 53.7 years.

Q192R polymorphism of paraoxonase 1 gene (including QQ, RR, and QR genotypes), was analyzed using the PCR-RFLP method. Based on the results obtained, there was no significant difference ($p>0.2$) in the frequencies of the genotypes between

TABLE 1: Frequency of genotypes in the total population.

			Polymorphism		Total
			QR	QQ	RR
CAD	Number	28	66	8	102
	%	27.5%	64.7%	7.8%	100.0%
Normal	Number	11	28	8	47
	%	23.4%	59.6%	17.0%	100.0%
Total	Number	39	94	16	149
	%	26.2%	63.1%	10.7%	100.0%

patients and control group (27.5% vs. 24.4% for QR genotype, 64.7% vs. 59.6% for QQ genotype and 7.8% vs. 17% for RR genotype) (Table 1).

We also categorized the subjects by ethnicity and determined the frequency of genotypes in the Arab and the Lur ethnic groups. The results of statistical analysis by ethnicity are presented in Table 2 and Table 3.

Genotype frequencies of PON1 Q192R polymorphism in the Lur and the Arab ethnic group are illustrated in Figure 1. According to Figure 1, there was no significant difference in genotype frequencies in the Lur ethnic group, While in the Arab ethnic group, the prevalence of QQ genotype was significantly higher ($p<0.05$) in the patients than controls (71.2% vs. 14.3). The prevalence of RR in the patients was significantly ($p<0.05$) lower than controls (4.3% vs. 42.9%).

DISCUSSION

Various studies around the world have been conducted on the association between PON1 gene polymorphism and CAD, and different results have been obtained in various populations. So these findings cannot be generalized to the Iranian population, and

TABLE 2: ANOVA results for the Arab ethnic group.

			Polymorphism			
			QR	QQ	RR	Total
Arab ethnic group	CAD	Number	15	42	2	59
		%	25.4%	71.2%	3.4%	100.0%
	Normal	Number	6	2	6	14
		%	42.9%	14.3%	42.9%	100.0%
	Total	Number	21	44	8	73
		%	28.8%	60.3%	11.0%	100.0%

TABLE 3: ANOVA results for the Lur ethnic group.

			Polymorphism			
			QR	QQ	RR	Total
Lur ethnic group	CAD	Number	9	18	3	30
		%	30.0%	60.0%	10.0%	100.0%
	Normal	Number	3	7	1	11
		%	27,3%	63.6%	9.1%	100.0%
	Total	Number	21	12	25	4
		%	28.8%	29.3%	61.0%	9.8%

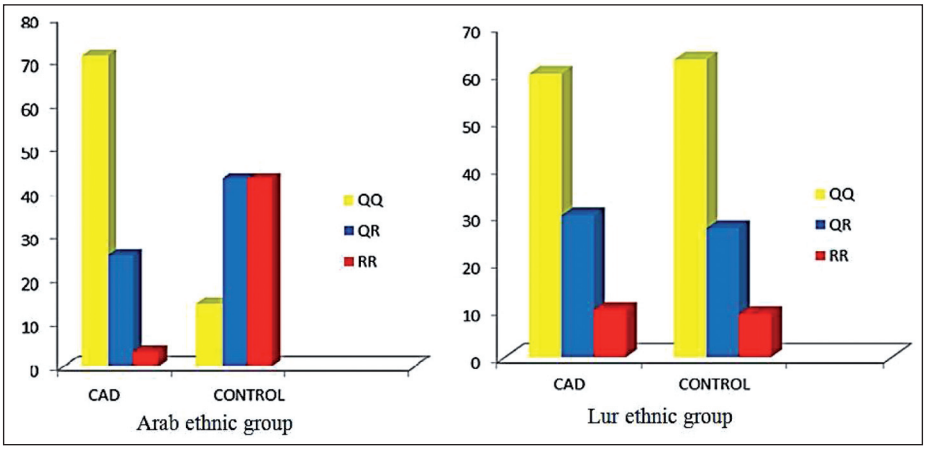

FIGURE 1: Genotype frequencies of PON1 Q192R polymorphism in the Arab and the Lur ethnic groups.

separate studies are needed to be carried out in Iran. A meta-analysis in 2016 indicated a significant association between Q192R PON1 polymorphism and CAD in Asian and African populations, but no association in Europe and America.[11] Our study demonstrated no association between CAD and Q192R PON1 polymorphism. The result was similar to the outcome of Taskiran et al.'s survey conducted in Turkey.[12] In agreement with our study, a meta-analysis by Lawlor et al. in Britain showed no association between PON1 and CAD in Caucasians.[13]

In contrast to our finding, the meta-analysis of China in 2012 found that the decrease in PON1 activity was associated with an increased risk of CAD.[14] We also categorized the population by ethnicity and analyzed the frequency of genotypes in each ethnic group separately. In Lur ethnicity, the result was similar to that of the total population, and there was no significant difference in frequency of genotypes between the patients and controls. In the Arab ethnic group, there was a significant association between Q192R polymorphism and CAD. QQ genotype frequency was significantly higher, and RR genotype was significantly lower in patients than in controls. This result is compatible with the results of the study conducted in the United States in 2008 but in contrast to the results of studies in Turkey by Ozkok, E et al in 2008, India by Agrawal, S et al. in 2009, Saudi Arabia by Hassan, M et al. in 2013 and Iran (Kermanshah) by Vaisi-Raygani, A et al. in 2011, in which, RR genotype has been reported as a risk factor for CAD.[15-19] It should be noted that there are limitations in the present study, among which limited sample size, especially in ethnic groups, therefore, it is recommended to increase the sample size and study separately on ethnic groups.

CONCLUSION

In the present study, there was no significant correlation between PON1 Q192R polymorphism and CAD in the whole population regardless of ethnicity, but in a separate study in the Arab race, QQ genotype was significantly higher, and RR genotype was significantly lower in CAD group. Therefore, QQ genotype can be considered as a risk factor and RR genotype as a protective factor for CAD in the Arab race.

Source of Finance

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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: *Ebrahim Heidari Sardabi;* ***Design:*** *Nastaran Mosadegh Rad;* ***Control/Supervision:*** *Nastaran Mosadegh Rad;* ***Data Collection and/or Processing:*** *Nastaran Mosadegh Rad,*

Ebrahim Heidari Sardabi; ***Analysis and/or Interpretation:*** *Nastaran Mosadegh Rad, Ebrahim Heidari Sardabi;* ***Literature Review:*** *Nastaran Mosadegh Rad;* ***Writing the Article:*** *Nastaran Mosadegh Rad;* ***Critical Review:*** *Nastaran Mosadegh Rad;* ***References and Fundings:*** *Ebrahim Heidari Sardabi;* ***Materials:*** *Ebrahim Heidari Sardabi.*

REFERENCES

1. Krishnan MN. Coronary heart disease and risk factors in India-on the brink of an epidemic? Indian Heart J. 2012;64(4):364-7. [Crossref] [PubMed] [PMC]
2. Firdaus M, Asbury JM, Reynolds DW. A new paradigm of cardiovascular risk factor modification. Vasc Health Risk Manag. 2005;1(2):101-9. [Crossref] [PubMed] [PMC]
3. Yang C, Wang X, Ding H. Is coronary artery disease a multifactorial inherited disorder with a sex-influenced trait. Med Hypotheses. 2008;71(3):449-52. [Crossref] [PubMed]
4. Sayols-Baixeras S, Lluís-Ganella C, Lucas G, Elosua R. Pathogenesis of coronary artery disease: focus on genetic risk factors and identification of genetic variants. Appl Clin Genet. 2014;7:15-32. [Crossref] [PubMed] [PMC]
5. Ghattas A, Griffiths HR, Devitt A, Lip GY, Shantsila E. Monocytes in coronary artery disease and atherosclerosis: where are we now? J Am Coll Cardiol. 2013;62(17):1541-51. [Crossref] [PubMed]
6. Podrez EA. Antioxidant properties of high-density lipoprotein and atherosclerosis. Clin Exp Pharmacol Physiol. 2010; 7(7):719-25. [Crossref] [PubMed] [PMC]
7. Ansell BJ, Fonarow GC, Fogelman AM. The paradox of dysfunctional high-density lipoprotein. Curr Opin Lipidol. 2007;18(4):427-34. [Crossref] [PubMed]
8. Rosenblat M, Vaya J, Shih D, Aviram M. Paraoxonase 1 (PON1) enhances HDL-mediated macrophage cholesterol efflux via the ABCA1 transporter in association with increased HDL binding to the cells: a possible role for lysophosphatidylcholine. Atherosclerosis. 2005;179(1):69-77. [Crossref] [PubMed]
9. Chistiakov DA, Melnichenko AA, Orekhov AN, Bobryshev YV. Paraoxonase and atherosclerosis-related cardiovascular diseases. Biochimie. 2017;132:19-27. [Crossref] [PubMed]
10. Birjmohun RS, Vergeer M, Stroes ES, Sandhu MS, Ricketts SL, Tanck MW, et al. Both paraoxonase-1 genotype and activity do not predict the risk of future coronary artery disease; the EPIC-Norfolk Prospective Population Study. PloS One. 2009;4(8):e6809. [Crossref] [PubMed] [PMC]
11. Hernández-Díaz Y, Tovilla-Zárate CA, Juárez-Rojop IE, González-Castro TB, Rodríguez-Pérez C, López-Narváez, et al. Effects of paraoxonase 1 gene polymorphisms on heart diseases: systematic review and meta-analysis of 64 case-control studies. Medicine (Baltimore). 2016;95(44):e5298. [Crossref] [PubMed] [PMC]
12. Taşkiran P, Cam SF, Sekuri C, Tüzün N, Alioğlu E, Altıntaş N, et al. The relationship between paraoxanase gene Leu-Met (55) and Gln-Arg (192) polymorphisms and coronary artery disease. Turk Kardiyol Dern Ars. 2009;37(7):473-8. [PubMed]
13. Lawlor DA, Day IN, Gaunt TR, Hinks LJ, Briggs PJ, Kiessling M, et al. The association of the PON1 Q192R polymorphism with coronary heart disease: findings from the British Women's Heart and Health cohort study and a meta-analysis. BMC Genet. 2004;5(1):17. [Crossref] [PubMed] [PMC]
14. Zhao Y, Ma Y, Fang Y, Liu L, Wu S, Fu D, et al. Association between PON1 activity and coronary heart disease risk: a meta-analysis based on 43 studies. Mol Genet Metab. 2012;105(1):141-8. [Crossref] [PubMed]
15. Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X, Schmitt D, et al. Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. JAMA. 2008;299(11):1265-76. [Crossref] [PubMed] [PMC]
16. Ozkök E, Aydin M, Babalik E, Ozbek Z, Ince N, Kara I. Combined impact of matrix metalloproteinase-3 and paraoxonase 1 55/192 gene variants on coronary artery disease in Turkish patients. Med Sci Monit. 2008;14(10):CR536-42. [PubMed]
17. Agrawal S, Tripathi G, Prajnya R, Sinha N, Gilmour A, Bush L, et al. Paraoxonase 1 GENE polymorphisms contribute to coronary artery disease risk. Indian J Med Sci. 2009;63(8):335-44. [Crossref] [PubMed]
18. 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(1-2):121-8. [Crossref] [PubMed]
19. Vaisi-Raygani A, Ghaneialvar H, Rahimi Z, Tavilani H, Pourmotabbed T, Shakiba E, et al. Paraoxonase Arg 192 allele is an independent risk factor for three-vessel stenosis of coronary artery disease. Mol Biol Rep. 2011;38(8):5421-8. [Crossref] [PubMed]