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Original Article

The Association between Apolipoprotein E Genotypes and Serum Malondialdehyde Level with End-Stage Renal Disease

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Abstract

Background: The Apolipoprotein E (ApoE) polymorphism plays an important role in the pathophysiology of end-stage renal disease (ESRD). Additionally, ApoE may contribute to the progression of oxidative stress. Thus, this study aimed to determine the ApoE gene polymorphism and evaluate the malondialdehyde (MDA) level in ESRD patients and healthy individuals.

Methods: The present cross-sectional study was conducted at 2010 at Kermanshah University of Medical Sciences (Kermanshah, Iran). The study population comprised ESRD patients (n=136) and healthy individuals (n=137). The MDA level was assessed using high-performance liquid chromatography (HPLC), and the frequencies of ApoE gene alleles were analyzed using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). The data were analyzed using Statistical Package for Social Sciences (SPSS), version 13. The significant differences of ApoE genotypes in case and control groups were assessed using Pearson's Chi square tests, and two-tailed Student's tests. A logistic regression model was used to calculate the odd ratio. $P<0.05$ was considered statistically significant.

Results: According to the results, ESRD patients had a higher frequency of the E_2/E_3 genotype than the healthy group ($P<0.001$). The results indicated that E_3/E_4 genotype frequency in the patients' group was higher than that of the control group ($P=0.026$). Furthermore, the E_3/E_2 (OR=5.7, 95% CI=2.68-12.14) ($P<0.001$) and E_3/E_4 (OR=1.57, 95% CI=1.05-2.34) ($P=0.029$) genotypes were found to increase the risk of ESRD. Moreover, the MDA level in ESRD patients was higher than the healthy individuals ($P<0.001$). The patients with E_3/E_2 ($P<0.001$) and E_3/E_4 ($P<0.001$) genotypes had a higher level of MDA than the control group.

Conclusion: According to the findings, patients with ESRD had higher genotypes of E_3/E_2 and E_3/E_4, which suggests a higher risk of developing ESRD.

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Keywords ● Kidney Failure, Chronic ● Apolipoproteins E ● Malondialdehyde ● Polymorphism

What's Known

- Apolipoprotein E gene polymorphism has a crucial role in lipid metabolism, oxidative stress, and the progression of end-stage renal disease.

What's New

- Our findings revealed that the prevalence of ApoE2 and ApoE4 genotypes were higher in patients with end-stage renal disease in Kermanshah, Iran.
- Based on our findings, the Malondialdehyde (MDA) level in patients with E_2 and E_4 genotypes was significantly higher than the control group.

Introduction

Chronic kidney disease (CKD), which affects 8-16% of the population, is regarded as a serious public health issue. The CKD

eventually leads to end-stage renal disease (ESRD). ESRD patients frequently require dialysis and renal replacement therapy.[1, 2] The pathogenesis of CKD is influenced by age, hypertension, inflammation, diabetes, oxidative stress, and environmental factors such as increasing calorie intake and altered lipid metabolism.[3]

As previously stated, altered lipid metabolism plays a key role in ESRD pathogenesis, and previous studies indicated that ApoE gene polymorphism was correlated with the risk of ESRD.[4, 5] ApoE is a 34-kDa protein, and its gene is located on chromosome 19. There are three alleles for the ApoE gene including ε2, ε3, and ε4, which ultimately lead to ApoE2, ApoE3, and ApoE4 protein isoforms, respectively.[6, 7] ApoE has several functions, including lipid metabolism, cell proliferation, differentiation, and tissue injury repair.[8] The ApoE genotypes are associated with the risk of several diseases such as cardiovascular diseases, Alzheimer's, and kidney impairment.[9] According to previous studies, ApoE2 significantly increased the risk of ESRD, while ApoE4 was associated with an increased risk of cardiovascular diseases.[10, 11] These findings could be attributed to the weak affinity of ApoE2 allele for its receptor and subsequent hyperlipidemia, or changes in ApoE4 allele stability.[12, 13] The other mechanisms include reduced clearance of very-low-density lipoprotein and chylomicron by ApoE2, binding of ApoE2 to extracellular glycosaminoglycans, and association of ApoE2 with lipoprotein glomerulopathy.[11, 14, 15] On the other hand, it was observed that ApoE alleles may contribute to the progression of oxidative stress, which plays a vital role in ESRD pathogenesis.

Oxidative stress conditions are caused by either an overproduction of reactive oxygen species (ROS), an inadequate antioxidant defense system, or both. Proteins, DNA, carbohydrates, and lipids are all damaged by oxidative stress.[16] The malondialdehyde (MDA), which is generated during lipid peroxidation of fatty acids in oxidative stress conditions, is considered an oxidative stress marker.[16, 17] Previous studies showed that ApoE4 genotypes elevated the risk of oxidative stress and played a vital role in its progression.[18-20] Given the importance of ApoE alleles and oxidative stress in the progression of ESRD and due to the association of ApoE alleles with oxidative stress, this study was designed to investigate the association of ApoE gene alleles and MDA level as an oxidative stress marker with the risk of ESRD in Kermanshah, Iran.

Materials and Methods

The present cross-sectional study was conducted in 2010 at the Department of Laboratory Sciences, School of Allied Medical Sciences, Kermanshah University of Medical Sciences (Kermanshah, Iran). Using the formula below, The minimum sample size in each group was calculated to be 135 (95% confidence level, 90% power, and d=20%).

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \times (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

The case group consisted of 136 hemodialysis patients with ESRD who received maintenance hemodialysis at least three times per week at the Nephrology Unit of Imam Reza Hospital, affiliated with Kermanshah University of Medical Sciences (Kermanshah, Iran). Based on the exclusion criteria of this study, the patients with antioxidant therapy or infectious diseases were excluded from the study. The control group consisted of 137 healthy individuals, who were matched for age and sex. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences (IR.KUMS.REC.1395.294) and was carried out in agreement with the principles of the Helsinki Declaration. All the participants were informed about the goals of the research, and written informed consent was obtained from the patients before participation. Then, their primary information such as age, sex, and blood pressure level were obtained and recorded.

Genotyping

The genomic DNA was extracted from 7 mL of whole blood by the phenol-chloroform extraction method. The ApoE gene was amplified by the polymerase chain reaction (PCR) method and using the specific primers forward: -5-TCC AAG GAG CTG CAG GCG GCG CA-3- and the reverse: -5- ACAGAATCCGC CCCGGCCTGGTACACTGCCA-3-. The product was checked using 1% agarose and digested by the Cfol restriction enzyme. The digested fragments were electrophoresed on a 12% polyacrylamide gel, and separated bands were revealed by ethidium bromide. The DNA fragments were specified and compared against DNA molecular weight marker. For each genotype, the obtained fragment patterns were as follows: E_3/E_3 one fragment (91 bp), E_3/E_4 two fragments (81 bp & 91 bp), and E_2/E_4 two fragments (91 bp & 71 bp).

Chemical Analysis

The MDA level, as a marker of oxidative stress condition, was measured using an Agilent Technologies 1200 Series, high-performance liquid chromatography (HPLC) system (Agilent

Corp., Germany) and EC 250/4.6 Nucleodur 100-5 C18ec column (Macherey- Nagel, Duren, Germany).

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) version 13.0, and all data were expressed as Mean±SEM. The frequencies of ApoE genotypes were determined by genotype counting. The Chi square test was used to analyze the statistical differences in ApoE genotype frequencies between ESRD patients and healthy individuals. For evaluating the relative risk of disease, the odd ratio (OR) was estimated using the logistic regression model. For quantitative data, the two-tailed Student's tests were used. For all analyses, a P value<0.05 was considered statistically significant.

Results

The demographic characteristics of the participants are summarized in table 1. As shown in table 1, there was no statistical difference in the sex or age means (P=0.4, P=0.27, respectively) between the case and control groups. The serum MDA level in ESRD patients (2.04±0.4 µmol/L) was higher than the control group (1.1±0.33 µmol/L; P<0.001) (table 1 and figure 1).

The frequencies of E_3/E_3, E_2/E_3, E_3/E_4, and E_4/E_4 genotypes in both the case and control groups are shown in table 2. The findings showed there were significant differences in ApoE genotype frequencies between the case and control groups (Chi square=27.37, P<0.001). Although these differences were not statistically significant, the results suggested that the E_3/E_3 and E_4/E_4 genotype frequencies in the control group (healthy individuals) were higher than those of the case group (ERSD patients). In addition, the analysis revealed that the frequencies of E_2/E_3 (Chi square=23.42, P<0.001) and E_3/E_4 (Chi square=4.98, P=0.026) genotypes differed significantly from the reference genotype (E_3/E_3 genotype). The odd ratio of the E_2/E_3 genotype showed that this genotype increased the

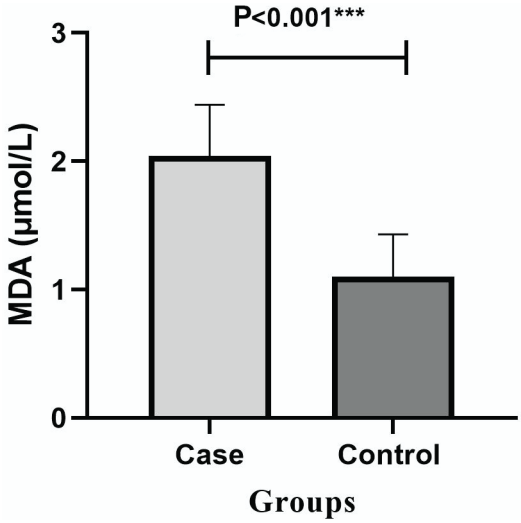

Figure 1: The MDA level was assessed in case (ESRD patients) and control (healthy individuals) groups. Data were analyzed using two-tailed Student's tests and represented as mean±SD. P<0.05 was considered statistically significant. The MDA level in the case group significantly increased compared with the control group (P<0.001).

Table 1: Demographic characteristics of participants in the present study

Variable		Case (mean±SD)	Control (mean±SD)	P value
Age (year)		58±13.3	55.7±7.3	0.27
Sex n (%)	Male	89 (65.44)	87 (73.5)	0.4
	Female	47 (34.55)	50 (36.5)	
MDA (µmol/L)		2.04±0.4	1.1±0.33	<0.001

Case: Patients with ESRD; Control: Healthy individuals; Data were analyzed using two-tailed Student's tests and represented as mean±SD. P<0.05 was considered significant.

Table 2: Distributions of ApoE genotypes in case and control groups

Apo E genotype	Case (n=136)	Control (n=137)	P value	OR (95% confidential interval)
E_3/E_3, N (%)	74 (56.9)	111 (82.8)	<0.001	-
E_3/E_2, N (%)	38 (29.2)	10 (7.5)		
E_3/E_4, N (%)	18 (13.8)	11 (8.2)		
E_4/E_4, N (%)	0 (0)	2 (1.5)		
E_3/E_3 (reference group), N (%)	74 (66.1)	111 (91.7)	<0.001	5.7 (2.68-12.14),
E_3/E_2, N (%)	38 (33.9)	10 (8.3)		
E_3/E_3 (reference group), N (%)	74 (66.1)	111 (91.7)	0.026	1.57 (1.05-2.34)
E_3/E_4, N (%)	18 (19.6)	11 (9.0)		

Case: Patients with ESRD; Control: Healthy individuals; The significant differences of ApoE genotypes in case and control groups were assessed using the Chi square test. The logistic regression model was used to calculate the odd ratio. P<0.05 was considered significant.

Table 3: Mean concentration of Malondialdehyde (MDA) in E_3/E_3, E_3/E_2 and E_3/E_4 genotypes in case and control groups

ApoE genotype	Case (n=133) (mean±SD)	Control (n=137) (mean±SD)	P value
E_3/E_2 MDA (µmol/L)	n=74 2.09±0.42	n=111 2.06±10.21	0.98
E_3/E_4 MDA (µmol/L)	n=38 1.93±0.34	n=10 1.12±23	<0.001
E_3/E_4 MDA (µmol/L)	n=18 2.10±0.42	n=11 1.29±0.34	<0.001

Case: Patients with ESRD; Control: Healthy individuals; Data were analyzed using two-tailed Student's tests and represented as mean±SD, P<0.05 considered as significant.

incidence of ESRD by 5.7 times (95% CI=2.68-12.14, P<0.001). The findings also indicated that the odd ratio for E_3/E_4 genotype was 1.57 (95% CI=1.05-2.34, P=0.029).

MDA levels in ESRD patients were higher than the control group (2.09±0.42 vs 2.06±10.21 µmol/L) when comparing people with the E_3/E_3 genotype in the two study groups. However, the difference was not statistically significant (table 3).

As presented in table 3, the MDA levels in ERSD patients with E_2/E_3 (1.93±0.34 µmol/L) and E_3/E_4 (2.10±0.42 µmol/L) genotypes were statistically higher than those of their healthy peers with E_2/E_3 (1.12±0.23 µmol/L) and E_3/E_4 (1.28± 0.34 µmol/L) genotypes (P<0.001).

Discussion

The findings of the present study indicated that ESRD patients had higher frequencies of the E_2/E_3 and E_3/E_4 genotypes than healthy individuals, which suggests a higher risk for ESRD. In addition, the MDA levels in ESRD patients were significantly higher than the healthy controls. These findings raise the possibility that Apo E genotypes contribute to the development of ESRD and oxidative stress.

Previous research found that ApoE2 and ApoE4 alleles increased the risk of ESRD; while, the E_3 allele frequency was lower in ESRD patients.[6, 20, 21] In the present study, we found that the frequency of the E_2 allele was significantly higher in the ESRD patient group, and E_2/E_3 genotype increased the risk of ESRD up to 5.7 times. In contrast, the E_3 allele frequency was lower in the ESRD patients group. Furthermore, the results showed that the E_3/E_4 genotype was significantly higher in ESRD patients than the control group, which meant that having this genotype raised the risk of ESRD up to 1.56 times. In light of these findings, it seemed possible that the ApoE genotypes influence the risk of ESRD. In a study on 107 Japanese patients with glomerulonephritis, the E_2 and E_4 alleles frequencies were significantly higher in patients than the control group. However, the E_3 allele frequency was lower in the patients' group.[22] On the other hand, Onuma and colleagues found no correlation between ApoE genotypes and the incidence of diabetic nephropathy in patients with diabetes mellitus.[23] Lahrach and others conducted a study on 109 ESRD patients and 97 healthy individuals as the control group. They reported that the E_4 allele may be associated with an increased risk of developing ESRD. They also found that E_4 and E_2 alleles may involve in lipid metabolism and accelerate the development of cardiovascular diseases.[4] Hubacek and colleagues conducted a study on 995 ESRD patients and 6242 controls in the Czech Republic and found that the ApoE2 allele was significantly associated with the risk of ESRD.[24] The increased risk of ESRD brought on by ApoE2 and E_4 alleles might be associated with a weak affinity of ApoE2 for its receptor, which leads to abnormal lipid metabolism. The ApoE4, on the other hand, may be involved in abnormal lipid metabolism due to its decreased stability.[12, 13]

The other mechanism that may contribute to the development of ESRD is oxidative stress, and previous studies reported that ApoE alleles might be associated with the risk of oxidative stress. In line with the results of a previous study,[25] we observed that the MDA level, as a marker of oxidative stress, in ESRD patients with E_3/E_2 and E_3/E_4 genotypes were significantly higher than the healthy individuals with similar genotypes.

The main limitation of the present study was our inability to measure the levels of reactive oxygen species to have a more accurate estimation for assessing oxidative stress conditions.

Conclusion

The present study indicated that ESRD patients had higher (statistically significant) E_2/E_3 and E_3/E_4 genotype frequencies than the healthy subjects. It can be concluded that these genotypes were associated with an increased risk of ESRD. Moreover, the higher MDA level in the patient group might indicate the presence of oxidative stress in ESRD patients.

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Authors' Contribution

D.P, A.V.R, F.B, and S.A: Contributed to designing the work; data acquisition; data analysis and interpretation of data; drafting and critically revision. All authors read and approved the final version of the manuscript and agree with all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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