Original Article

Serum Level of Apolipoprotein E and its gene polymorphism in Iraq Alzheimer Patients

Noora Wael Rasheed

Department of Medical Laboratory Techniques, Al Rafidain University College
Email: noora.waal@ruc.edu.iq

Abstract

Introduction: Apolipoprotein E (ApoE) is a circulating lipoprotein which governs lipid metabolism in the body. It is considered a serious genetic determinant of Alzheimer's disease (AD). **Objective:** The main goal of this study was to study any association between ApoE polymorphism with cholesterol and other lipids in Alzheimer patients in Bagdad, Iraq. **Methods:** In this case-control study, 75 male patients with average age of 65 years from Baghdad medical city in Iraq and 50 healthy males as controls were recruited. Serum lipid level was tested enzymatically and DNA from all study participants was extracted to detect ApoE polymorphism using PCR. **Results:** The cholesterol level was found significantly increased. Similar results were observed with the levels of triglycerides in patients i.e. 418.43±11.95mg/dl. Furthermore, data revealed non-significant difference in the genotypic and allelic frequencies of the ApoE gene/ rs13333144 SNP in patients and controls. Also, both patients and controls showed a strong correlation between ApoE genotype and cholesterol levels. In addition, there was a lower level of apolipoprotein in the patient group as compared to the control group. Thus, the ApoE gene might play an important role in the development of AD in Iraqi patients, hence, studies investigating lipid levels and the risk of Alzheimer's disease should take ApoE status into account.

Keywords: Apolipoprotein E, polymorphism, Alzheimer's disease, ApoE genotype.

Introduction

Apolipoprotein E, a 299-amino-acid lipoprotein, encodes the APOE gene (ApoE). The molecular mass of this lipoprotein is 34 kDa.[1] As a major cholesterol transporter in the brain, it participates in lipid transport and its metabolism and modulates brain damage recovery. Also, it migrates across tissues and cells through ApoE receptors and proteins involved in lipid transport and lipolysis.[2,3] It is known that the ApoE gene has three frequent polymorphisms i.e. e2, e3 and e4, with three homozygous (e2/e2, e3/e3, e4/e4) and three heterozygous genotypes (e3/e2, e4/e2, e4/e3). Each of these genotypes result in a unique amino acid alteration in the ApoE protein. Amongst these, the most common allele is e3, followed by e4 and e2 with rates varying between different populations.[4] Alzheimer's disease (AD) is a fatal neurodegenerative illness that affects around 26 million people worldwide and causes memory loss, cognitive decline and dementia over time.[5,6] Extracellular and intracellular accumulation of amyloid β (Aβ), neural or synapse loss, brain atrophy and inflammatory processes are the most common pathological features of this disease.[7] From 2000 to 2008, fatalities due to AD increased by 66% which made it the sixth leading cause of death in the United States. According to estimates, 13% of adults over 65 years of age and 45% of those over 85 years are expected to suffer from this disorder. To detect, prevent and control this disease among vulnerable population, its pathogenesis and etiology must be understood.[1] The human Apolipoprotein E (ApoE) gene which is found on chromosome 19, has been found to be associated with the onset of AD in various studies conducted over the previous half-century.[8] Since AD occurs episodically, scientists are investigating molecular warning elements which could be its contributing factors. However, further research is required to fully understand the possible link between ApoE and AD progress.

Address for Correspondence: Department of Medical Laboratory Techniques, Al Rafidain University College
Email: noora.waal@ruc.edu.iq

Submitted: 09th October, 2022 **Received:** 17th October, 2022
Accepted: 15th November, 2022 **Published:** 22nd December, 2022

Access this article online

Quick Response Code:

Website: www.jnsbm.org

DOI: 10.4103/jnsbm.JNSBM_13_2_8

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Rasheed N W. Serum Level of Apolipoprotein E and its gene polymorphism in Iraq Alzheimer Patients. J Nat Sc Biol Med 2022;13:142-146

The current study sought to identify and compare the serum levels of ApoE gene and lipids between AD patients and healthy controls. Furthermore, the study also investigated the possible effect of polymorphisms in the ApoE gene on Alzheimer's progression.

Materials and Methods

Subjects

In total, 125 subjects were included in this study comprising of 75 patients and 50 healthy controls. The patients were recruited from several hospitals in Baghdad Medical City, Iraq. All the patients were male with an average age of 65 years. Moreover, the healthy individuals included in the study had no history of systemic disorders. Because all the patients were male, therefore, the control samples involved in the study were also male to avoid any ambiguity in the study process. All the samples were taken between the years of 2021 and 2022.

Lipid analysis

Blood samples were collected from the study participants while fasting to determine cholesterol and triglycerides levels using an automated analyzer and an enzyme-based colorimetric ELISA.

Blood sampling and ApoE genotyping

Blood samples from the study subjects were drawn from their veins and transferred to the EDTA-containing tubes for DNA extraction and purification according to the manufacturer's instructions (Quick-gDNATM MiniPrep Zymo). For molecular analysis, the DNA was eluted and kept at -20°C. Genomic DNA was produced with primers specific to the ApoE gene (Forward primer: 5'-CTGACGTACTAAGCGTTCAGTC-3' and Reverse primer: 5'-TAGCTAGCACGTCAGTCAAGCT-3'). Multi Gene Opti Max gradient thermal cycler (Labnet/USA) was used for this purpose.

The single nucleotide polymorphisms (SNP_s) in ApoE gene were identified by SNP genotyping using a Real-Time PCR System (Real-time thermal cycler Sa Cycler-96, Sacace Bio technologies, Italy). The reaction mixture for real-time PCR was prepared according to the manufacturer's instructions. This included 10µl of TaqMan® master mix (2x), 0.5µl of SNP genotyping assay working stock (20x), 10.4µl each of forward and reverse primer, 100ng of template DNA and nuclease free water to bring the final volume up to 10µl. The cycling parameters of custom SNP genotyping assay were adapted according to the information provided by the manufacturer.

Statistical Analysis

The Statistical Analysis System- SAS[9] application was utilized to analyze different research parameters. The analysis of variance test (ANOVA) and the T-test were used to compare mean values while the Chi-square test was used to compare percentages. The p-value less than 0.05 was considered statistically significant.

Results

Basic demographic characteristics of the study subjects

An overview of the demographic characteristics of the study subjects is provided in Table 1. In the current study, the distribution between the patient and control group showed a significant prevalence based on age and levels of cholesterol and triglycerides. As it is evident from results, there was a significant increase in cholesterol levels in patients i.e. 429.73±10.39 as compared to that in the control group i.e. 154.48±4.13 (P= 0.001). Likewise, level of triglycerides in patient group was found to be 418.43±11.95 while it was 144.28±3.74 in the healthy group (P≤0.001).

Table 1: Results of factors studied in patients and controls

Factors	Patients	Controls	P-value
Numbers (Mean±SE)	75 (60.0%)	50 (40.0%)	1.5×10^{-12} **
Age (years)	29.95±0.89	54.72±0.90	4.07×10^{-38} **
Cholesterol level	429.73±10.39	154.48±4.13	2.9×10^{-42} **
Triglycerides level	418.43±11.95	144.28±3.74	6.23×10^{-37} **

** (P≤0.001).

ApoE genotype and allele frequencies

The genotypic distribution of the SNPs in both patients and controls is summarized in Table 2. The genotypic and allelic frequencies of the ApoE gene/ rs13333144 SNP in patients and controls were found to be non-significant.

Table 2: Genotype and Allele frequency of ApoE gene/ rs13333144 SNP in Patients and Control groups

Genotype (rs13333144)	Patients No. (%)	Control No. (%)	P-value	Chi-Square (χ^2)
TT	5 (6.67)	4 (8.0)	0.778	0.080
TC	26 (34.67)	18 (36.0)	0.878	0.023
CC	44 (58.67)	28 (56.0)	0.768	0.087
Allele (rs13333144)		**Frequency**		
T	0.24	0.26	0.720	0.129
C	0.76	0.74	0.720	0.129

NS: non-significant

As shown in Table 3, a strong correlation was found between ApoE genotypes and cholesterol levels in patients and the control group. In other words, the level of cholesterol in all ApoE genotypes in the patient's group was found to be significantly high as opposed to the control group. In the TT genotype, the mean cholesterol level in patient's group came out to be 402.25±34.4 which is significantly higher as compared to the cholesterol level of control group i.e. 165.25± 14.7 (P≤0.001). Similar significant results were observed for the genotypes TC and CC also (P≤0.001). Likewise, the level of triglycerides in all ApoE genotypes was significantly higher in the patient's group as compared to the control group. Table 3 shows that in the TT genotype, the level of triglycerides in the patient's group is 436.75±29.39 which is significantly higher in comparison to the control group's triglycerides level i.e. 142.75±15.77 (P≤0.001). Similarly, for TC and CC genotypes, the mean level of triglycerides in patients was found to be 406.15±19.31 and 428.03±16.25 respectively (P≤0.001). Interestingly, the Apolipoprotein levels were found significantly reduced in all genotypes in patients as compared to controls (P≤0.001). As it is evident from Table 3, the mean level of Apolipoprotein in patients with genotype TT is 14.75±4.27 while its level in controls with same genotype is higher i.e. 69.75±16.92. Same pattern was observed in patients and controls with TC and CC genotypes.

Table 3: Relationship between Genotype of *ApoE* gene and Cholesterol, Triglycerides and Apolioprotein levels of patients and controls.

		Genotype (SNP: rs13333144)			
		TT	TC	CC	Least Significant Difference (LSD value)
Cholesterol	Patients	402.25±34.44	433.73±17.91	431.57±13.93	380.18
	Controls	165.25±14.70	157.72±7.14	150.86±5.46	33.0
	Probability	0.0002 **	6.06×10^{-13} **	6.05×10^{-13} **	
Triglycerides	Patients	436.75±29.39	406.15±19.31	428.03±16.25	107.35
	Controls	142.75±15.77	138.50±5.40	148.21±5.31	29.78
	Probability	0.00002 **	6.05×10^{-13} **	6.06×10^{-13} **	
Apolioprotein	Patients	14.75±4.27	14.50±6.11	15.50±6.05	9.01
	Controls	69.75±16.92	55.0±7.78	54.75±7.45	9.23
	Probability	6.06×10^{-13} **	6.05×10^{-13} **	6.06×10^{-13} **	

** (P≤0.001)

In addition, Table 4 provides information about the Apolipoprotein levels in patients and controls and their correlation. The Apolipoprotein level was found remarkably high in the control group than in the patient group with mean level of 56.04±9.26 in former group and 15.25±6.04 in latter group (P≤0.001).

Table 4: Apolioprotein level in patients and control groups

	Patients	Control	P-value
Apolioprotein level (Mean±SE)	15.25±6.04	56.04±9.26	3.78×10^{-58} **

** (P≤0.001)

Discussion

The ApoE gene, which regulates and controls cholesterol and triglycerides metabolism, has been immunochemically linked to Alzheimer's disease senile plaques, vascular amyloid and neurofibrillary tangles.[10,11] Besides controlling blood cholesterol levels, ApoE protein plays a key role in Aβ deposition, aggregation and clearance.[12] Additionally, the ApoE e4 allele has been found to be associated with late-onset familial AD.[13] In comparison to ApoAI and B tests, analyses on serum ApoE concentrations are hardly performed in analytical laboratories. Although genetic variation at the ApoE locus is a substantial driver of serum ApoE concentration, a significant amount of variability remains unexplained, implying that additional genetic and environmental factors are key determinants of serum ApoE concentration. In this study, the effects of ApoE and age on lipid concentrations and the effects of ApoE gene polymorphism on AD development ApoE concentration were examined to provide useful information for clinical trials. Based on the different genotypes of the ApoE gene, remarkable difference was found in cholesterol levels between AD patients and normal controls. The cholesterol level in AD patients was approximately 2.5 times higher than in normal people across all genotypes studied (TT, TC, CC). Similar results were obtained with triglycerides levels where they were approximately three times higher in AD patients than in normal people. Previously, Schiele et al. conducted a study to demonstrate similarity between the ApoE and triglycerides levels in patients and controls but found no significant difference in ApoE concentration. However, the triglycerides levels were found significantly higher in patients.[14] On the contrary, some studies found no association between triglycerides levels and Alzheimer's disease.[15,16] The precise mechanism by which ApoE genotypes and lipids contribute to the progression of Alzheimer's disease is unknown. Each

ApoE genotype has been shown to have a distinct affinity for lipoproteins and also, it has been reported that ApoE variants affect cholesterol metabolic pathways centrally and peripherally.[17] A study reported high cholesterol in late life to be associated with a decreased risk of dementia.[18] Similar to the results of current research, a study observed low total serum cholesterol among AD patients with e2 carriers than those with e4 carriers.[19] Moreover, it appears that ApoE's affinity for ApoA is influenced not only by variation type, but also by whether it is related to lipids or not.[20]

According to a meta-analysis study, the prevalence of ApoE genotype differs among Alzheimer's patients, and it plays a crucial role in the disease's occurrence.[21] The ApoE polymorphism can cause a variety of expected and unexpected health problems, so it should be taken into account continuously. Furthermore, research investigations are likely to be more or less biased and artifactual than observational surveys.

Conclusion

The current study reveals that the ApoE gene may play a crucial role in the development of AD in Iraqi patients. In demographic studies, ApoE status must be taken into consideration when examining the association between lipid levels and the risk of AD. Even though the ApoE polymorphism is a well-studied genetic risk factor for this disease, the majority of people in some areas do not have this genotype. To establish other genetic and environmental risk markers, more research involving larger specimens will be required in the future.

References

1. Liu M, Bian C, Zhang J, Wen F. Apolipoprotein E gene polymorphism and Alzheimer's disease in Chinese population: a meta-analysis. Sci Rep. 2014; 4: 4383. doi: https://doi.org/10.1038/srep04383.
2. Emrani S, Arain HA, DeMarshall C, Nuriel T. APOE4 is associated with cognitive and pathological heterogeneity in patients with Alzheimer's disease: a systematic review. Alzheimers Res Ther. 2020; 12(1): 141. doi: https://doi.org/10.1186/s13195-020-00712-4.
3. Koutsodendris N, Nelson MR, Rao A, Huang Y. Apolipoprotein E and Alzheimer's Disease: Findings, Hypotheses, and Potential Mechanisms. Annu Rev Pathol. 2022; 17: 73-99. doi: https://doi.org/10.1146/annurev-pathmechdis-030421-112756.
4. Yassine HN, Finch CE. APOE Alleles and Diet in Brain Aging and Alzheimer's Disease. Front Aging Neurosci. 2020; 12: 150. doi: https://doi.org/10.3389/fnagi.2020.00150.
5. Solana C, Tarazona R, Solana R. Immunosenescence of Natural Killer Cells, Inflammation, and Alzheimer's Disease. Int J Alzheimers Dis. 2018; 2018: 3128758. doi: https://doi.org/10.1155/2018/3128758.
6. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002; 297(5580): 353-6. doi: https://doi.org/10.1126/science.1072994.
7. Mathys H, Davila-Velderrain J, Peng Z, et al. Single-cell transcriptomic analysis of Alzheimer's disease. Nature. 2019; 570(7761): 332-37. doi: https://doi.org/10.1038/s41586-019-1195-2.
8. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. Mol Neurodegener. 2019; 14(1): 32. doi: https://doi.org/10.1186/s13024-019-0333-5.
9. SAS J. Statistical Analysis System, v.10.0.2. 2012: Available from: https://support.sas.com/downloads/browse.htm?fil=&cat=536.
10. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology. 1993; 43(8): 1467-72. doi: https://doi.org/10.1212/wnl.43.8.1467.
11. Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G. Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. Nat Rev Neurol. 2019; 15(9): 501-18. doi: https://doi.org/10.1038/s41582-019-0228-7.
12. Husain MA, Laurent B, Plourde M. APOE and Alzheimer's Disease: From Lipid Transport to Physiopathology and Therapeutics. Front Neurosci. 2021; 15: 630502. doi: https://doi.org/10.3389/fnins.2021.630502.
13. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A. 1993; 90(5): 1977-81. doi: https://doi.org/10.1073/pnas.90.5.1977.
14. Schiele F, De Bacquer D, Vincent-Viry M, et al. Apolipoprotein E serum concentration and polymorphism in six European countries: the ApoEurope Project. Atherosclerosis. 2000; 152(2): 475-88. doi: https://doi.org/10.1016/s0021-9150(99)00501-8.
15. Zhao B, Shang S, Li P, et al. The gender- and age-dependent relationships between serum lipids and cognitive impairment: a cross-sectional study in a rural area of Xi'an, China. Lipids Health Dis. 2019; 18(1): 4. doi: https://doi.org/10.1186/s12944-018-0956-5.
16. Vasantharekha R, Priyanka HP, Swarnalingam T, Srinivasan AV, ThyagaRajan S. Interrelationship between Mini-Mental State Examination scores and biochemical parameters in patients with mild cognitive impairment and Alzheimer's disease. Geriatr Gerontol Int. 2017; 17(10): 1737-45. doi: https://doi.org/10.1111/ggi.12957.
17. Wingo TS, Cutler DJ, Wingo AP, et al. Association of Early-Onset Alzheimer Disease With Elevated Low-Density Lipoprotein Cholesterol Levels and Rare Genetic Coding Variants of APOB. JAMA Neurol. 2019; 76(7): 809-17. doi: https://doi.org/10.1001/jamaneurol.2019.0648.

18. Mielke MM, Zandi PP, Sjögren M, et al. High total cholesterol levels in late life associated with a reduced risk of dementia. Neurology. 2005; 64(10): 1689-95. doi: https://doi.org/10.1212/01.wnl.0000161870.78572.a5.
19. Isbir T, Agaçhan B, Yilmaz H, et al. Apolipoprotein-E gene polymorphism and lipid profiles in Alzheimer's disease. Am J Alzheimers Dis Other Demen. 2001; 16(2): 77-81. doi: https://doi.org/10.1177/153331750101600207.
20. Kloske CM, Wilcock DM. The Important Interface Between Apolipoprotein E and Neuroinflammation in Alzheimer's Disease. Front Immunol. 2020; 11: 11-754. doi: https://doi.org/10.3389/fimmu.2020.00754.
21. Ward A, Crean S, Mercaldi CJ, et al. Prevalence of apolipoprotein E4 genotype and homozygotes (APOE e4/4) among patients diagnosed with Alzheimer's disease: a systematic review and meta-analysis. Neuroepidemiology. 2012; 38(1): 1-17. doi: https://doi.org/10.1159/000334607.