International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Association of ApoE Gene Polymorphism and Severity of Injury in Traumatic Brain Injury

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Abstract

Traumatic Brain Injury (TBI) is a complex form of acquired brain damage resulting from external force, causing structural and functional changes in the brain. The study aims to assess the association of apoE gene polymorphism with the severity of injury (GCS) following TBI. The research was conducted as a cross-sectional study over three years at a tertiary care hospital in Mangalore, involving TBI patients and healthy controls. Ethical considerations and convenient sampling are implemented, with blood samples collected within 48 hours for genetic polymorphism analysis. The study examined ApoE gene genotype frequencies in traumatic brain injury (TBI) patients and healthy controls, revealing that in the TBI population, the most prevalent genotype was e3/e3 (71.8%), followed by e3/e4, e2/e3, e2/e4, e2/e2, and e4/e4. Among healthy controls, e3/e3 was the predominant genotype (64.34%). Notably, the e4/e4 genotype was absent in healthy controls. There were no significant deviations from Hardy-Weinberg equilibrium for ApoE (p=0.77) gene polymorphisms among TBI patients or healthy controls, suggesting genetic equilibrium. ApoE3 was identified as the common wild-type allele, while ApoE2 and ApoE4 were recognized as mutant alleles. The association between ApoE gene polymorphism and Glasgow Coma Scale (GCS) scores on admission was assessed under various models, including additive, e2 vs non-e2, e3 vs non-e3, and e4 vs non-e4. The results did not indicate a significant association between ApoE gene polymorphisms and the severity of injury, as assessed by GCS scores, except for a notable association with Pulse (p=0.019). In conclusion, apoE gene polymorphism may not significantly contribute to the severity of injury.

Keywords: ApoE gene, TBI, Glasgow coma scale, severity of injury

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1. Introduction

Traumatic brain injury (TBI) is a diverse form of acquired brain damage caused by external force, leading to changes in brain function and structure [1]. Often referred to as the "silent epidemic," TBI isa major global health concern, affecting individuals of all backgrounds, ages, and genders. Due to the wide range of injury mechanisms, force dynamics, and patient factors (including genetics and protective measures), each TBI case is unique, resulting in varying recovery outcomes. As such, there is a pressing need for the development of effective prognostic models and treatment strategies using admission data, with ongoing research efforts in this field.

The 2017 Commission noted that TBI would remain a leading cause of injury-related fatalities and disabilities until 2030. Globally, TBI's annual incidence is estimated to be between 27 to 69 million cases [2]. Over 2 million people in Europe are hospitalized for TBI each year, resulting in approximately 82,000 deaths. In the USA, 153 individuals die daily due to TBI, with an annual hospitalisation rate of 2.8 million. India faces a significant TBI challenge, especially among the youngand productive population. Epidemiological data suggests approximately 1.6 million TBI cases annually, leading to 200,000 deaths and a need for rehabilitation programs for nearly 1 million people.

Pathophysiology designates TBI under two levels of injury. The mechanical damage caused immediately by trauma is denoted as the primary injury. In contrast, the gradual physiologicalshortcomings of the initial injury lead to secondary brain injury, which evolves eventually. The process includes a cascade of events, like hypercapnia resulting in increased blood flow to the brain, a rise in intracranial pressure and edematous displacement of brain tissue or herniation, consequently leading to further complications such as ischemia, excitotoxicity, metabolic acidosis, oxidative stress, inflammation and apoptosis. Studies on gene expression have demonstrated that several genes are crucial in the pathophysiology of secondary brain injury.

Understanding the genetic factors influencing traumatic brain injury (TBI) outcomes is a growing areaof research. APOE is intricately tied to cholesterol metabolism and manifests in three isoforms: E2, E3, and E4, each differing by a single amino acid. APOE is predominantly produced by astrocytes in the brain andplays a crucial role in maintaining neuronal health. While certain studies have associated the variant of apoE with unfavourable TBI outcomes, others have failed to establish a significant connection. This relationship is still a subject of exploration, particularly with limited data available from Indian studies on this topic. The objective of the study was to assess the association of apoE Gene Polymorphism with the severity of injury (GCS) after Traumatic Brain Injury.

2. Methodology

The study was designed as a cross-sectional study conducted at the Neurosurgery Inpatient Department and the Casualty of Justice K S Hegde Charitable Hospital in Deralakatte, Mangalore, with additional laboratory work at the Central Research Laboratory, KSHEMA. The study spanned three years, from February 2019 to January 2022, focusing on patients aged 18-70 with traumatic brain injuries and healthy donors seeking medical aid in the tertiary care hospital. Inclusion criteria for cases include a specific age range, presentation within 48 hours, and willingness to participate. The patients are categorized based on the Glasgow Coma Scale into Mild, Moderate, and Severe TBI groups. Controls consist of normal healthy donors without trauma. Exclusion criteria for both cases and controls involve various neurological and psychiatric disorders. Clinical evaluations encompass demographic details, injury specifics, and various clinical features on admission such as GCS, blood pressure, CT-Brain findings, and more, to comprehensively study the impact of traumatic brain injury.

The study adheres to ethical considerations with approval granted by the Central Ethics Committee of NITTE (Deemed to be University), indicated by approval numbers NU/CEC/2019/0250, NU/CEC/2020/0338, and NU/CEC/ 2021/154 on specific dates. Convenient sampling was employed for subject selection. Blood samples, collected within 48 hours of injury, were obtained from traumatic brain injury (TBI) patients and healthy donors for genotyping. TBI patients' blood samples undergo genetic polymorphism analysis, with 2 ml of EDTA whole blood stored at -20º C. Additionally, 2 ml of plain vial blood is centrifuged for serum, and stored at -20º C for biochemical parameter analysis. The laboratory analysis involved DNA isolation from blood using a modified Miller et al. protocol, quantified by a Nano-drop spectrophotometer, with DNA stored at -20°C for subsequent analysis. The PCR RFLP was carried out using suitable primers and restriction enzymes (Table 1).

3. Results

The study participants ranged from 17 to 72 years of age. Most subjects were males (n=65), 76.5% of the total population, whereas females (n=20) covered 23.5%. The study subjects were categorised as per the severity score (GCS) under three categories on admission. Maximum subjects were allotted in the Mild TBI group (n=39, 45.9%), followed by Moderate TBI (n=25, 29.4%) and Severe TBI (n=21, 24.7%). The mean age of our study subjects was 39.05 ±14.035, with the mean GCS score noted on admission10.99± 4.0. (Table 2). The genotype frequencies of the ApoE gene were examined in both traumatic brain injury (TBI) patients and healthy controls. In the TBI population, the most common genotype was e3/e3 (71.8%),followed by e3/e4, e2/e3, e2/e4, e2/e2, and e4/e4 (table 3). Among healthy controls, the predominant genotype was e3/e3 (64.34%), followed by e2/e3, e3/e4, e2/e4, and e2/e2 (table 4). No individualswith the e4/e4 genotype were found among the healthy control subjects.

There was no significant deviation of observed gene frequencies for ApoE (p=0.77) and TP53 (p=0.98) gene polymorphisms from the expected as shown by HWE among TBI patients or healthy controls (Table 4), suggesting that alleles were in equilibrium. ApoE 3 was the common wild-type allele, whereas ApoE2 and ApoE4 are mutant alleles in the ApoE gene. The association of ApoE gene polymorphism with GCS scores on admission was assessed under various models (Table 5). ApoE gene polymorphisms under an additive model, e2 vs non-e2, e3 vs non-e3, and e4 vs non-e4 models did not show any significant association with the severity of injury as per GCS scores (p>0.05). Except for the Pulse (p=0.019), none of the clinical features recorded on admission exhibited significant association with ApoE gene polymorphism.

4. Discussion

The ApoE gene polymorphism was categorized into six different genotypes (e2/e2, e2/e3, e2/e4, e3/e3, e3/e4, and e4/e4) and three dichotomized groups (e2 vs non-e2, e3 vs non-e3, and e4 vs non-e4). The analysis showed that ApoE gene polymorphism was not significantly associated with age, gender, and mechanism of injury in any of the tested models. Furthermore, the association between ApoE gene polymorphism and the severity of injury, assessed using the Glasgow Coma Scale (GCS) score on admission, was also examined. However, no significant associations were found between the individual genotypes of ApoE and trichotomized GCS scores or dichotomized GCS groups (mild vs moderate + severe, and mild + moderate vs severe).

Specifically, there was no significant association between the e2 isoform and GCS scores or between the e3 isoform and GCS scores. Similarly, the presence or absence of the e4 isoform was not significantly associated with different GCS score groups. Overall, the study did not find any significant associations between ApoE gene polymorphism and demographic characteristics or the severity of traumatic brain injury, as measured by the GCS score.

Table 1: Primer sequence, PCR, and PCR-RFLP for ApoE gene

SNP Id	Primers	Annealing temperature	PCR Product size	Restriction enzyme	ApoE genotype	Fragment size AflIII	Fragment size HaeII	Amino acid change
rs429358 rs7412	Forward- 5'ACTGACCCCGGTG GCGGAGGAGACGCG TGC3'	65°C	318bp	AflIII HaeII	ε2/ ε2 ε3/ ε3 ε4/ ε4	231bp 231bp 295bp	267bp 232bp 232bp	Cys112,Cys158 Cys112, Arg158 Arg112,Arg158
	Reverse- 5'TGTTCCACCAGGG GCCCCAGGCGCTGG CGG3'				ε2/ ε3	231bp	267bp 232bp	Cys112,Cys158,Arg158
					ε2/ ε4	295bp 231bp	267bp 232bp	Cys112,Cys158,Arg112,Arg158
					ε3/ ε4	295bp 231bp	232bp	Cys112, Arg112. Arg158

Table 2: Baseline characteristics of subjects with traumatic brain injury

Variable	N	Mean ±SD/ Median (IQR)	Min, Max
Age	85	39.05 ±14	16, 72
GCS	85	10.99±4	3, 15
systolic bp	83	128± 20.8	110, 140
diastolic bp	82	78.46 ±11.26	70, 90
pulse(bpm)	83	83±15.4	60, 140
Rotterdam CT Brain Total	85	2.5±0.92	1,6

Table 3: Pattern of ApoE gene polymorphism among TBI patients and healthy control subjects

ApoE gene polymorphism	Cases N=85		Control N=115	
	N	%	N	%
e2/e2	1	1.2%	2	1.73%
e2/e3	8	9.4%	18	15.65%
e2/e4	2	2.4%	6	5.21%
e3/e3	61	71.8%	74	64.34%
e3/e4	12	14.1%	15	13.04%
e4/e4	1	1.2%	0	0%

Table 4: Hardy-Weinberg Equilibrium for ApoE gene polymorphism

ApoE gene polymorphisms		Cases	Allele frequency	Control	Allele frequency
e2/e2	Observed	1	p=0.07	2	p =0.122
	Expected	0	q=0.84	2	q= 0.787
e2/e3	Observed	8	r=0.09	18	r =0.091
	Expected	10		22	
e2/e4	Observed	2		6	
	Expected	1		3	
e3/e3	Observed	61		74	
	Expected	59		71	
e3/e4	Observed	12	$\chi 2$= 1.74	15	$\chi 2$= 2.49
	Expected	14		16	
e4/e4	Observed	1	p=0.88	0	p=0.77
	Expected	1		1	

Table 5: Association of injury severity (GCS) on admission with ApoE gene polymorphism

ApoE gene polymorphism	GCS on Admission											
	Mild	Moderate	Severe	χ^2	p	Mild	Moderate + Severe	χ^2	p	Mild + Moderate	Severe	χ^2	p
e2/e2	0	0	1	Fishers	0.824	0	1	Fishers	0.815	0	1	Fishers	0.52
e2/e3	4	2	2	exact test		4	4	exact test		6	2	exact test	
e2/e4	1	0	1			1	1			1	1		
e3/e3	27	20	14			27	34			47	14		
e3/e4	6	3	3			6	6			9	3		
e4/e4	1	0	0			1	0			1	0		
e2	5	2	4	1.237	0.539	5	6	0.001	0.976	7	4	0.923	0.33
Non-e2	34	23	17			34	40			57	17		
e3	37	25	19	Fishers	0.311	37	44	Fishers	0.866	62	19	Fishers	0.23
Non-e3	2	0	2	exact test		2	2	exact test		2	2	exact test	
e4	8	3	4	0.797	0.671	8	7	0.407	0.523	11	4	0.038	0.84
Non-e4	31	22	17			31	39			53	17		

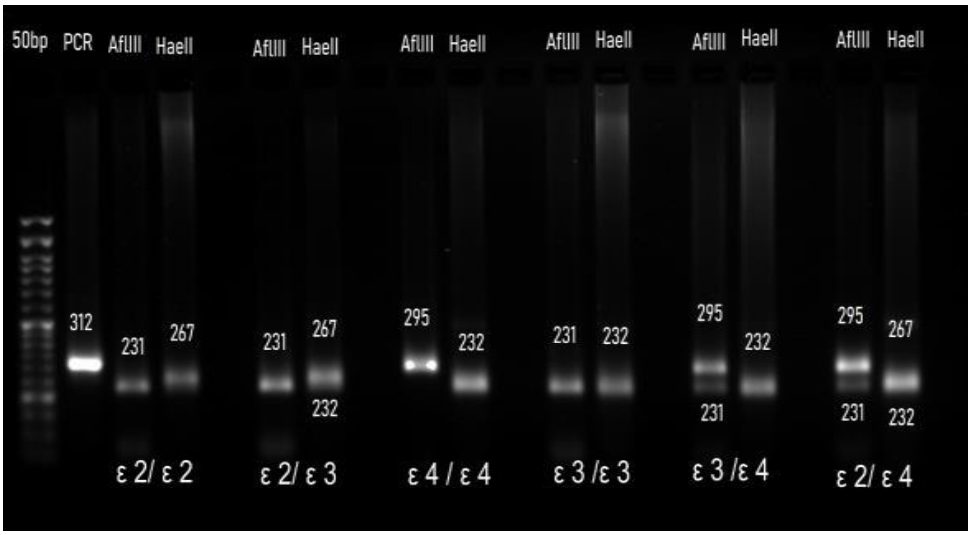

Fig 1: Electrophoresis gel image patterns of ApoE gene after digestion with AflIII and HaeII restriction enzymes

Production of Apolipoprotein E (Apo E) by macrophages has been demonstrated to facilitate nerve regeneration post-sciatic nerve transection in rats [3]. However, a growing body of evidence indicates a connection between Apo E and unfavourable outcomes following traumatic brain injury (TBI) [4-6]. Numerous studies generally agree, suggesting a detrimental impact of the E4 variant on head injury outcomes [7-10], although Teasdale and colleagues did not observe an overall worse outcome in E4 carriers [11]. The E4 allele has been associated with impaired cognitive functions and increased neurobehavioral disturbances [12,13]. The detrimental effects of the E4 allele may involve larger intra-cerebral hematomas, worse contusions, and ischemic brain damage post-TBI [14,15].

Several studies support the association between the possession of the ε4 allele and negative neuropsychiatric outcomes, including an increased risk of late-onset Alzheimer's disease and intracerebral haemorrhage [16]. ApoE2 is suggested to have neuroprotective effects compared to E4, while the toxic effects of ApoE4 are well-documented neurochemically [17,18]. The unique structure of the E4 isoform, containing an arginine replacing a cysteine at residue 112, is associated with domain interaction, resulting in aberrant cleavage and the release of neurotoxic fragments. APOE4 inhibits neurite outgrowth and enhances the release of pro-inflammatory mediators from microglia, contributing to cellular apoptosis. Given these pathological functions, it is hypothesized that TBI patients with the ε4 allele may experience more severe injuries with greater secondary damage and impaired recovery.

Contradictory results exist among studies examining global outcome measures in TBI. Lichtman and colleagues reported worse outcomes for APOE4+ patients on the Functional Independence Measure in severe TBI cases [19], while Mejia and colleagues found an association between APOE3+ genotypes and improved scores at 6 months [20]. Other studies reported no significant difference in outcomes based on APOE genotype, including studies on mild-moderate TBI [21,22] and the Transforming Research and Clinical Knowledge in TBI (TRACK-TBI) cohort [23]. Factors such as mortality, post-traumatic seizure occurrence, early clinical deterioration, and the need for decompressive craniectomy have shown varying associations with APOE genotypes across different studies [24-27]. The G219T promoter SNP in the APOE gene promoter region has also been linked to worse outcomes [28].

5. Conclusion

ApoE3 was the predominant genotype in Indian TBI patients. There was no significant association between the ApoE gene polymorphism and the severity of injury in terms of the Glasgow coma scale.

Conflicts of interest

None

Funding

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