Original Article

Lack of association between TNF-α gene polymorphisms at position -308 A, -850T and risk of simple febrile convulsion in pediatric patients

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Abstract

Background: Febrile convulsions (FCs), occurring between 6 months and 6 years of age is the most common seizure disorder during childhood. The febrile response is thought to be mediated by the release of pyrogenic cytokines, such as tumor necrosis factor and interleukin-1 (IL-1). There is a significant relationship between genetic components for susceptibility of FCs and different report mutation. We investigated association between two polymorphisms in the tumor necrosis factor (TNF)-α promoter region (G-308A, C-850T) and FCs in the southwest area of Iran. **Materials and Methods:** In this matched case–control study, 100 patients with febrile convulsion as case group and 130 healthy children as control group were enrolled in the study. Peripheral blood samples were collected and DNA was extracted by standard phenol–chloroform method. The genotype and allele frequencies of TNF- α polymorphisms in case and control groups were determined by using PCR-RFLP (polymerase chain reaction restriction fragment length polymorphism) method. Statistical analysis was done using Chi-square test. **Results:** The average age of case and control groups were 3.4 ± 1.4 and 3.4 ± 1.2 years, respectively. There was no significant difference between age and sex in both the groups ($P > 0.05$). A family history of febrile convulsion was detected in 44% of patients. Moreover, the simple febrile convulsion was detected in 85% of the case group. **Conclusion:** RFLP analysis of TNF- α promoter region polymorphisms, considering $P = 0.146$ and $P = 0.084$ for G-308A and C-850T, respectively, showed no correlation between TNF- α polymorphisms and predisposition to simple febrile, based on the kind of convulsion (atypical and simple febrile convulsion). We found a significant relation between genotype distribution of G-308A and atypical febrile convulsion in case group ($P = 0.04$). A significant correlation between genotype distribution of G-308A and atypical febrile convulsion in the case group was found, but there was no correlation between TNF- α polymorphisms at positions of –308A, and 850T and predisposition to simple febrile convulsion. Further studies are needed to understand clinical usefulness of this correlation.

Key words: Atypical febrile convulsion, polymorphism, simple febrile convulsion, TNF-α gene

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INTRODUCTION

Fever is a typical response to a variety of exogenous pyrogens, with infection being the most common cause. Fever induced by systemic injection of lipopolysaccharide, an endotoxin from the cell membrane of gram-negative bacteria, enhances susceptibility to seizures provoked by both pentylenetetrazole and kainic acid.[1]

This finding suggests that the exogenous pyrogens can facilitate seizures with different underlying mechanisms. Exogenous pyrogens induce fever by interactions with cytokines, which are referred to as endogenous pyrogens. These include interleukins 1β, 6, and 8 (IL-1β, IL-6, IL-8) and also tumor necrosis factor-α.[2] A number of exogenous substances are capable of evoking fever. All these exogenous pyrogens induce the production and release of immunoregulatory proteins, which are generally termed cytokines. It has been proposed that circulating cytokines are the endogenous mediators of fever in response to administration of an exogenous pyrogen. The first set of observations relate to the appearance of a number of cytokines in the bloodstream more or less simultaneously to the development of fever after injection of an exogenous pyrogen, such as lipopolysaccharide (LPS), from the cell wall of gram-negative bacteria. Depending on the route and injected dose of LPS, tumor necrosis factor alpha (TNF-α is the first cytokine, which appears in the circulation followed by trace of IL-1β and high amount of IL-6 as well as other cytokines.[3]

Febrile convulsions (FCs) are the most common type of seizures in children between 6 months and 6 years with incidence of 2%–5%.[4] FCs are defined as any seizure that occurs in response to a febrile stimulus in the absence of meningitis, encephalitis, serum electrolyte imbalance and other acute neurologic illness.[5] Simple FCs are generalized tonic–colonic seizures lasting less than 15 min followed by a brief postictal period of drowsiness and occurs only once in 24 h, whereas atypical febrile convulsion, lasting more than 15 min has focal features and multiple recurrences within 24 h.[6] Febrile seizures are predominantly brief and 4%–16% have focal features according to epidemiologic studies.[7] The prevalence of febrile seizures is between 3% and 8% in children up to 7 years of age.[8] Variation in prevalence relates to differences in case definitions, geographic variation and cultural factors. Febrile seizures result from the combination of genetic and environmental factors.[9] Of children with febrile seizures, 24% have a family history of febrile seizures and 4% have a family history of epilepsy. Thirty percent of children have recurrent febrile seizures during subsequent illnesses.[10] Risk factors for recurrence include onset before 18 months, lower temperature close to 38°C, shorter duration of fever (<1 h) before the seizure and a family history of febrile seizures. Of children who have all these risk factors, 76% will have a recurrence of febrile seizure compared with 4% without risk factors.[11] Although polygenic inheritance is usual, a small number of families exist in whom the inheritance of febrile seizures is autosomal dominant and several chromosomal loci and a few genes have been identified.[12] A strong familial history of FCs in siblings and parents suggests a genetic predisposition.[13,14] Recently, FCs have been suggested to be a gene-related disease.[15] FCs in children are the result of complex interactions between the immune inflammatory processes, cytokine activation, and genetic factors.[16] Linkage analysis, mainly of large families, revealed 7 putative febrile seizure loci, chromosomes 8q,[17] 19p,[15] 2q,[18] 5q[19,20] 18q,[21] and 3p,[20] which are at least partly associated with epilepsy or febrile seizures. Febrile seizures are mostly provoked by infections. A proinflammatory cytokine, IL-1β, play an important role in host defense during infections and act as endogenous pyrogen. The balance between proinflammatory (IL-1, TNF-α, IL-8, and IL-6) and anti-inflammatory (IL-1 receptor antagonist and IL-10) cytokines influence the regulation of infections and could, therefore, play a role in pathogenesis of febrile seizure.[22] A rapid rise of fever during an infectious disease can trigger seizure in individuals who are prone to febrile seizure.[5,22] Fever is mediated by the release of pyrogenic cytokines, such as TNF, IL-1, and IL-6 into the blood stream. TNF-α is a potent immunomediator and proinflammatory cytokine that has implicated in the pathogenesis of a large number of human disease.[23] The TNF-α gene is located on the short arm of chromosome 6 within the major histocompatibility complex, where genetic alternations in the TNF-α locus are now known to be involved directly in high TNF-α production.[24] The biological function of the TNF-α are diverse and complex, where on one hand it confers disease resistance and on the other causes pathologic complications.[24] On the other hand, TNF-α plays a contradictory role, which may be related to genetic polymorphisms in the gene regulating its production and effect.[25] In the acute situation, local production of TNF-α is clearly beneficial. It increases the expression of adhesion molecules on the vascular endothelium to allow immune cells, in particular, neutrophils and macrophages, to translocate to sites of tissue damage and infection. Furthermore, TNF-α activates phagocytes to engulf and clear infectious agents and cellular debris.[25] The induction of IL-1 and IL-6 production stimulated by TNF-Alpha leads to elevated temperature.[26] Several polymorphisms have

been identified inside the TNF-α promoter that among these variants, a polymorphism that directly affects TNF-α expression is located at nucleotide position -308. A single base polymorphism within the promoter of the gene for TNF-α results in 2 allelic forms, one in which guanine defines the common allele (TNFA1) and the other in which guanine is substituted by adenosine from the rare allele (TNFA2) at position -308.[25,26] The presence of the rare TNFA2 allele has been found to correlate with enhanced spontaneous or stimulated TNF-α production in both *in vitro* and *in vivo*.[27] TNF is a pyrogenic cytokine that acts directly on the set point of hypothalamus.[5] Also increased levels of TNF-α in the brain result in significant inhibition of seizures in mice.[28] Some findings indicate that TNF-α and IL-1β play a role in the very early sensitization of the central nervous system to convulsive activity after *Shigella dysenteriae* administration.[29] The interaction of these cytokines influences the level of fever and has a role in pathogenesis of FCs.[30] Cytokine genes may be related to cytokine expression and distinct alleles of cytokine genes, which have been discovered to be associated with different immune-inflammatory diseases.[30] It was showed that the IL-1 receptor antagonist allele I is associated with higher susceptibility to FCs.[31] On the other hand, carriage of a guanine (G) to adenine (A) transition at position -308 of the TNF-α promoter is associated with significantly increased TNF-α production and transcription.[32] Increased level of TNF-α induces inhibition of seizure in mice.[33] This data suggests that cytokine gene may have a role in susceptibility to FCs.[34] While there has been no published study examining the association between TNF-α promoter polymorphism and febrile convulsion, we designed a study to determine whether polymorphisms of the gene encoding TNF-α (308 and 850) are related to febrile convulsion susceptibility in our patients or not.

MATERIALS AND METHODS

This matched case–control study was conducted in Cellular and Molecular Research Center of Shahrekord University of Medical Sciences, Shahrekord, Iran. One hundred patients with diagnosis of FCs and 130 healthy children without fever and seizure disorder in the pediatric and emergency wards of Hajar Hospital were enrolled to the study. The study was carried out from April 2009 to April 2010 and was approved by the research ethics committee of university too. All parents of the patients included in this project, informed and signed consent form. The information regarding the demographic data, such as age, sex, and family history of FCs and history of recurrence and type of FCs were recorded. Febrile seizure was defined as a convulsion associated with a temperature of higher than 38.0°C not caused by an intracranial infection, toxin-induced bacterial gastroenteritis. We used the following criteria for the simple type of febrile seizure,[34] which are frequently employed in Japan: (1) negative family history of epilepsy, (2) negative past history of any disease with potential to cause brain damage, (3) age at the first febrile seizure between 6 months and 6 years of age, (4) duration of convulsion less than 20 min, (5) pattern of convulsions: generalized, bilateral symmetrical, or lacking focal symptoms, (6) no clustering of frequent convulsion within a short period, (7) postictal phase: uneventful and complete recovery without sequelae (eg, long lasting disturbance of consciousness, hemiplegic, aphasia, and dementia), and (8) interracially, neither obvious neurologic nor mental defects. Febrile seizure not in accord with any one of the above 8 items were considered as being of the complex type (atypical) family histories were obtained through pediatricians' interview with the parents. Forty-four patients had a positive family history of febrile seizure in near relatives, whereas the others were sporadic. There were simple febrile seizure in 85 patients and complex type in 15 patients. Recurrent febrile seizure was seen in 33 patients.

Laboratory methods

DNA was extracted from peripheral blood samples by using a standard phenol–chloroform method.[23]

By PCR-RFLP (polymerase chain reaction restrictive fragmented length polymorphism) method the G-308A and C-850T polymorphism, in the promoter of TNF-α gene were genotyped.[35-37]

The following primers were used in the determination of G-308A and C-850T polymorphism of the TNF-α promoter gene.

The primer for G-308A polymorphism is F-5′-GGGACACACAAGCATCAAGG-3′;

R-5′-AATAGGTTTTGAGGGCCATG-3′).

This variant (A at -308) creates an NCOI (MBI Fermentas, Lithuania) restriction site and can be differentiated by size on a 12% polyacrylamide gel. The primer for C-850T polymorphism[29] is:

F-5′-AAGTCGAGTATGGGGACCCCCCGTTAA-3′;

R-5′CCCCAGTGTGTGGCCAT ATCTTCTT-3′

The PCR product was digested with Hinc II (MBI Fermentas, Lithuania) restriction enzyme and subjected to 12% polyacrylamide gel electrophoresis.

Statistical analysis

The association of genotype distribution with study group and recurrence status was analyzed by Chi-square test. Furthermore, likelihood ratio test was done to investigate genotype distribution regarding the kind of convulsion. The data were analyzed using SPSS version 17 and $P < 0.05$ was considered as statistically significant.

RESULTS

Two hundred and thirty children (100 patients and 130 controls) enrolled in this study. The overall age of subjects was from 0.9 to 6 years with mean of 3.3 ± 1.3 years. There was no significant difference of age between the 2 groups ($P = 0.257$). One hundred twenty-nine (56.1%) of the subjects were girls with no difference between the 2 groups ($P = 0.113$). A positive history of febrile convulsion was detected in 44 (44%) patients in the case group and in 3 (2.3%) subjects in the control group ($P < 0.001$). The genotype distribution of G-308A and C-850T polymorphism based on study group are shown in Table 1. For the G-308A polymorphism, the frequency of homozygote GG was the highest and the frequency of the heterozygote GA was the lowest. The GG genotype was seen above 4 times more than AA. For the C-850T polymorphism, we did not find any TT genotype and the frequency of homozygote CC genotype was seen 22 times more than CT. However, no significant differences in genotype frequencies of both G-308A and C-850T polymorphism were found between patients and controls ($P > 0.05$). The distribution of genotype frequencies based on recurrence of seizures is shown in Table 2. In the case group, 33 (33%) of the patients had recurrent seizures. For both G-308A and C-850T polymorphisms, there was no significant relationship between genotype and recurrent seizures ($P > 0.05$). The kind of seizures is shown in Table 3. In the case group, 85 (85%) patients had simple febrile seizures and 15 (15%) had atypical seizures. For the G-308A polymorphism, there was a significant relationship between genotype and kind of seizures ($P < 0.05$). For the C-850T polymorphism, no significant relationship between genotype and kind of seizures was found ($P > 0.05$).

Table 1: Distribution of genotype frequencies based on the study group

Allele	Genotype	Frequency (%)	Group		*P* value
			Patients n (%)	Control n (%)	
	GG	175 (76.1)	70 (70)	105 (80.8)	
G-308A	GA	15 (6.5)	9 (9)	6 (4.6)	0.146
	AA	40 (17.4)	21 (21)	19 (14.6)	
C-850T	CC	220 (95.7)	93 (93)	127 (97.7)	0.083
	CT	10 (4.3)	7 (7)	3 (2.3)	

Table 2: Distribution of genotype frequencies based on recurrence seizures in the case group

Allele	Genotype	Recurrence		*P* value
		Yes n (%)	No n (%)	
	GG	26 (78.8)	44 (65.7)	
G-308A	GA	1 (3)	8 (11.9)	0.262
	AA	6 (18.2)	15 (22.4)	
C-850T	CC	32 (97)	61 (91)	0.275
	CT	1 (3)	6 (9)	

Table 3: Distribution of genotype frequencies based on the kind of convulsion in the case group

Allele	Genotype	Frequency (%)	Kind of convulsion		*P* value
			Atypical FC n (%)	Simple FC n (%)	
	GG	70 (70)	14 (93.3)	56 (65.9)	
G-308A	GA	9 (9)	0 (0)	9 (10.6)	0.040*
	AA	21 (21)	1 (6.7)	20 (23.5)	
C-850T	CC	93 (93)	14 (93.3)	79 (92.9)	0.956*
	CT	7 (7)	1 (6.7)	6 (7.1)	

FC, febrile convulsions. *Based on likelihood ratio test.

DISCUSSION

This study investigated febrile convulsion in a southwest area of Iran relating to TNF- α G-308A and C-850T promoter polymorphisms. The results suggest that the TNF-α gene 308G/A variants do not play a major role in susceptibility to febrile seizures. Also, no significant association was found between the genotype distribution of G-308A and C-850T polymorphism in the study group. There was no TT genotype allele in both control and patient groups in C-850T gene polymorphism. A significant association was found between genotype distribution of G-308A and atypical febrile convulsion based on the kind of convulsion in the patient group. However, this association was not found for G-850 gene polymorphisms. Cytokines are low molecular weight regulatory proteins secreted by white blood cells and various other cells in the body in response to a number of stimuli.[38] A number of cytokines play a significant role in the development of acute and chronic inflammatory responses. Cytokines have been shown to contribute to neuronal death, therefore they may participate in the epileptogen process.[39,40] TNF is a pyrogenic cytokine that acts directly on the hypothalamus to elevate the thermal set point. It also causes fever by inducing IL-1 production.[41] In previous studies of cytokines and febrile seizures, activation of the cytokine network has been shown but the exact role of cytokine in the pathogenesis of febrile seizures is not known.[42,43] Also TNF-α and IL-1β contents were increased in the whole brain tissue after the rats were kindled

by electrical stimulation of amygdale.[44] In addition, intraperitoneal TNF-α administration was followed by an increased susceptibility of amygdale to kindling.[45] Several single nucleotide polymorphisms of the TNF-α gene have been described. The guanine to adenine (GA) transition at position -308 of the TNF-α gene promoter region has been associated with enhanced TNF-α production.[46] As TNF-α is a primary mediator of inflammatory response, altered TNF-α level was also suggested to play a role in the pathogenesis of febrile seizures. Also some studies showed that 308G/A polymorphism of TNF-α gene is related to higher susceptibility for asthma, scaring glaucoma, increased mortality of meningococcal infection, and cerebral malaria.[47-50] In parasitic infections the -308A allele has been associated with a 4-fold increase in the risk for cerebral malaria and a 7-fold increase in the risk for development of neurologic consequences. This association was shown to be independent of the inheritance of HLA antigen.[51] A similar study confirmed that there was an association between 308A allele and cerebral malaria in Sri Lankan population.[52] Also the polymorphism in the human TNF-α may be important in the susceptibility or severity of diseases and in other inflammatory conditions. Another study showed that -308A allele was associated with the most severe outcome, mucocutaneous leishmaniasis.[53] In multiple-injured patients with severe sepsis the TNF-α allele acts as a predictor of severe post-traumatic sepsis and increased level of circulating TNF-α.[54] Alzheimer's disease (AD) is one of the most common types of chronic neurodegenerative diseases. The current findings suggested an association between (850C/T) polymorphism and the risk of developing AD.[55] No positive associations between TNF-α promoter haplotypes and AD in Italian population have been reported by Tedde *et al*.[56] Bas**e**d on animal studies, proinflammatory cytokines, such as TNF-α and IL-1Ra and IL-1α have been suspected to have a role in precipitating febrile seizure by influencing neuronal excitability that may result in linking of fever and seizure activity and suggest that cytokine gene may act as enhancer or attenuator of febrile convulsion susceptibility.[57] Regarding association between the human IL-1β (-511) gene polymorphism and susceptibility to febrile convulsion no association between the IL-1β polymorphism and an increased risk for FC had been reported.[58] In an associated study of single-nucleotide polymorphisms (SNPs) of IL-1β, IL-Ra, IL-6 promoter, IL-8, IL10, and TNF-α gene in patients with febrile seizures and healthy control group were showed that febrile seizures are not associated with IL-β exon 5, IL-6 promoter, IL-8, IL10, and TNF-α promoter gene polymorphisms, but they found that the IL-1Ra allele I is associated with a higher susceptibility for febrile seizures.[59] Also another investigator observed that the IL-1Ra allele I is associated with higher susceptibility to FCs.[29] However, Haspolat *et al*. found no significant effects of this polymorphism on febrile seizure in Turkish children.[60] In our previous study on Iranian population no significant association between IL-1Ra allele I and susceptibility to febrile convulsion was found.[61] Geographic and ethnic differences may partly account for this discrepancy in the results. In one study on functional single-nucleotide polymorphism of promoter region of IL-10 gene and resistance to febrile seizure, it was demonstrated that the IL-10 -592 C allele and -1082A/-819C/-592C (ACC) haplotype, were associated with increased production of IL-10[62] and this association may confer resistance to febrile seizures.[63] IL-10 is a multifunctional anti-inflammatory cytokine that inhibits the production of pro-inflammatory cytokines, including TNF-α.[64] The correlation of the G-308A allele incidence with atypical FCs as evidenced in this study, has never been reported before. However, further studies evaluating this correlation needs to confirm its usefulness as a marker of predicting susceptibility to atypical FCs.

CONCLUSION

Finally, the genetic basis for febrile seizure may not be related to a single genetic variant, but may be influenced by multiple genes acting synergistically with environmental factors to increase the likelihood of developing the disease. Genetic association studies offer a powerful approach to identify the multiple variants of small effects that modulate susceptibility to common, complex disease.[65] In the present genetic associated study, the potential bias caused by population stratification can be minimized by sampling and matching cases and controls from the same source population and geographic region.

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