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Does the imbalance between agonistic and antagonistic IL-1 play a role in progression of febrile convulsions?

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Abstract. – OBJECTIVE: Inflammation may play an important role in the etiopathology of febrile convulsions (FC). IL-1β is an important mediator of inflammation and fever is also important information of FCs. It is suggested that there may be a relationship between polymorphisms of IL-1β and FC. The aim of the present study is to investigate the polymorphic stituation of promoter region of IL-1β in two sites (-31 and -511) and assess the IL-1 RA VNTR polymorphisms in FC patients in comparison with healthy control groups.

MATERIALS AND METHODS: Fifty FC patients and 50 healthy controls (HC) were included in the study. DNA extraction was performed by QIAamp DNA Mini Kit from peripheral blood lymphocytes of all subjects. IL-1β promoter polymorphisms were analyzed by PCR-RFLP, IL-1 RA VNTR polymorphisms were analyzed by PCR-agarose gel electrophoresis.

RESULTS: Genotype distribution of IL-1β promoter region in position -31 was statistically different between FC patients and control groups. Allele I and allele II of IL-1 RA distribution were also statistically different in FC patients and healthy controls.

CONCLUSIONS: We have found a significant association between IL-1 RA allele distribution and FC and a poor correlation of T/C substitution at the -31 position of IL-1β promoter in FC. Further studies are needed to investigate the gene expression levels and polymorphic situation in same samples.

Key Words:
Febrile Convulsions, Interleukin-1β, Promoter, Polymorphism, IL 1-RA.

Introduction

The term of "Febrile Convulsion; FC" identifies any seizure that occurs in response to a febrile stimulus[1]. These convulsions are defined with a rapidly rising temperature and usually develop when the core temperature reaches 39°C or higher[1,2]. FCs are the most common type of seizures seen in children younger than 6 years of age and affect 2-5% of all children[1,2].

Fever, is an important element of this type of seizure but the exact role of fever in the initiation/formation of febrile convulsion is not clear[1]. Some studies indicate about the role of IL-1β, an important mediator of inflammation. Gatti et al[3] have shown the role of IL-1β in inducing the fever when administered into the lateral cerebral ventricles or peripherally. Enhanced neuronal excitability and decreased seizure treshold after the IL-1β release is demonstrated by Vezzani et al[4]. Dube et al[5] have shown that IL-1β receptor deficient mice were resistant to experimental febrile seizure and they also showed that high IL-1β doses induced seizure only in IL-1β receptor expressing mice.

IL-1β is produced as a 31 kd precursor protein from the IL-1 family complex and it requires cleavage by caspase-1 for its active form[6,7]. Regulation of IL-1β occurs via IL-1 RA that binds to IL-1 receptors and inhibits the binding of IL-1β[8]. Alleles of some polymorphisms have different effects on transcription of the reporter gene and thus, these polymorphisms may play role in the regulation of the protein. Substitution of T/C at the position -31, located in the TATA box motif in the promoter region of IL-1β, affect the binding of several transcription factors and affect the transcription activity of IL-1β[9]. Santilla et al[10] have shown an association between IL-RA allele 2 and IL-1β gene position at -511 and they also reported carriers of allele 2 at position -511 have elevated capacity to produce IL-1β *in vitro*.

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According to the knowledge about changes in the gene expression level of IL-1β due to nucleotide substitution in the promoter region of IL-1β gene and the antagonist role of IL-1 RA in the regulation of IL-1β level, we aimed to investigate the genotype and allele frequencies of IL-1β promoter (-31 and -511) and also compare allelic frequencies of IL-1 RA between FC patients and healthy controls.

Patients and Methods

We collected the genetic and demographic data of fifty children between 2010 and 2011 who attended to Pediatric Emergency service of Istanbul Medeniyet University, Goztepe Training and Research Hospital (Istanbul, Turkey) with history of fever over 39°C and accompanying convulsions. These children consisted the study group and were compared with age and gender matched healthy children as control group (n=50). For the study, we obtained the genotype and allele frequencies in all 50 patients as well as in 50 unrelated and ethnically matched controls. The control group was collected among the children of the hospital staff staying at the day-care center. The children with pre-existing febrile seizure history were excluded.

The study protocol was approved by the local Ethics Committees, and written informed consent was obtained from the parents of all participants.

DNA analysis of both groups were done. DNA extraction was carried out by QIAamp DNA Mini Kit (Qiagen, Germany) according to the instructions of the manifacturer. The genotypes of IL-1β promoter position -31 and position -511 polymorphisms were identified by PCR-RFLP under the conditions specified by Kira et al[11]. For -31 C/T genotyping, the 239 bp PCR products digested by *Alu I* restriction enzyme, allele C gave two fragments; 236 and 3 bp and allele T gave three fragments; 139, 97 and 3 bps. Genotyping of -511 were done by digestion of 305 bp PCR products with *Ava I* restriction enzyme, allele C gave two products; 189 and 116 bp, allele T remained intact without digestion. The genotypes of the IL-1 RA polymorphisms were identified by PCR and agarose gel electrophoresis, PCR conditions and genotyping methods done as stated by Serdaroglu et al[12]. Polymorphisms of IL-1 RA were assesed as IL-1RN1 (four repeats; 410 bp), IL-1RN2 (two repeats; 240 bp), IL-1 RN3 (five repeats; 500 bp), IL-RN4 (three repeats; 325 bp) and IL-1 RN5 (six repeats; 595bp). Primers and restriction endonucleases used for IL-1 β31, -511 and IL-1 RA are listed in Table I.

Statistical Analysis

Statistical analysis were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Chi-square test was used to test the significance of distribution of FC patients and HC. Descriptive statistics were expressed as count and percent. $p < 0.05$ was considered statistically significant.

Results

Thirty of the study group and 25 of the control group were male (60%; 50% respectively). The mean age of the study group was 9±50 months (min: 9 months; max: 58 months) in match with the mean age of the controls 10±53 months ($p > 0.05$). 11 of the study group had family history (22%). Five of them experienced febrile convulsion for the first time and their mean age was 11±15.2 months. The febrile convulsions lasted for at least 7 seconds (7 patients) and maximum 22 minutes (2 patients). Upper respiratory tract infection was diagnosed in 40.3%, acute otitis media in 16.8%, acute gastroenteritis in 11.3% and bronchopneumonia in 13.1% of the cases. The cause was undefined at the rest of the cases. Mainly they were simple seizures; yet at 1 of 2 patients who had febrile convulsion for 22 minutes and a complication occurred (post-convulsive hemiparesis).

Distribution of genetic polymorphisms and results of statistical analyses of IL-1β-31 (T/C), IL-1β-511 (C/T) and IL-1 RA VNTR polymorphisms in FC patients and healthy controls are summarized in Table II. The T allele homozygotes and C allele homozygotes were more frequent in patients than in control group at -31 position of IL-1β. Statistical analyses have shown that there wasn't any statistical difference between patients and control groups in terms of TT and CC genotypes ($\chi^2 = 2.9$; $p = 0.09$, $\chi^2 = 1.86$; $p = 0.17$ respectively). When distribution of T and C alleles were analyzed, a poor correlation was found between patients and controls ($\chi^2 = 3.93$; $p = 0.047$).

In contrast, there was no significant difference in allele frequency or genotype distribution between the patients and control groups at -511 position of IL-1β promoter.

In this study we have observed just allele I (four repeats; 410 bp) and allele II (two repeats; 240

Table I. Primers and restriction endonucleases used[11,12].

Forward	Revers	RES	
IL-1β31	5'AGAAGCTTCCACCAATACTC	5'AGCACCTAGTTGTAAGGAAG	AluI
IL-1β511	5'TGGCATTGATCTGGTTCATC	5'GTTTAGGAATCTTCCCACTT	AvaI
IL-1 RA	5'TCAGCAACACTCCTAT	5'TCCTGGTCTGCAGGTAA	

bp) for IL-1 RA. The IL-1 RA I/I homozygotes were more frequent in control group than patients and I/II heterozygotes were more frequent in patient group than control group. Statistical analyses revealed a statistical difference regarding I/I and I/II genotypes between two groups ($\chi^2 = 5.95$; $p = 0.015$ and $\chi^2 = 5.25$; $p = 0.02$).

Discussion

Multiple genetic pathways have been implicated in the pathogenesis of FC. In a previous study we conducted, we have determined a significant relation between MEFV gene mutations and FC[13]. This situation and role of IL-1β in inflammation and fever, led us to assess the IL-1β polymorphisms in FC. IL-1 pathway is an important mediator of fever that enhances and reduces the neuronal excitability[14] and FC usually occurs during a rapid rise of fever[1,14]. In the formation of FC the balance between proinflammatory and anti-inflammatory cytokines may have a critical role.

In this study we investigated two SNPs in the promoter region of IL-1β and variable tandem repeat polymorphism found in the IL-1 RA intron 2. There was a poor correlation in the distribution of IL-1β-31 genotypes and allele frequencies between FC patients and control group. This site is located within the promoter of the IL-1β gene. T allele may have a role in the increase of gene expression of IL-1β and this may a cause of IL-1β releated fever in FC patients. Chen et al[9] have shown that, binding protein profile of this site is strikingly different for allele T and allele C. Patikoglou et al[15] showed the better binding and transcriptional activity of the T allele to TATA binding protein (TBP) than the C allele. Hall et al[16] showed that two copies of C allele at -31 did not produce greater amounts of IL-1β protein when compared with the subjects carrying 1 or 2 copies of the T allele and this data is consistent with the results of Pociot et al[17] which showed C allele inhibits LPS-induced DNA-protein complexes. These results and our findings indicate that T/C substi-

Table II. Genotype distribution and allele frequencies of studied groups.

			Case (n=50) (%)	Control (n=50) (%)	χ^2	*p* value
-31 T/C	Genotypes	TT	15 (%30)	7 (%14)	3.73	0.05
		TC	30 (%60)	32 (%64)	0.17	0.68
		CC	5 (%10)	11 (%22)	2.68	0.10
	Alleles	T	60 (%60)	46 (%46)	3.93	0.047
		C	40 (%40)	54 (%54)		
-511 C/T	Genotypes	CC	15 (%30)	18 (%36)	0.41	0.52
		CT	30 (%60)	23 (%46)	1.97	0.16
		TT	5 (%10)	9 (%18)	1.33	0.25
	Alleles	C	60 (%60)	59 (%59)	0.21	0.89
		T	40 (%40)	41 (%41)		
IL-1 RA	Genotypes	I/I	23 (%46)	36 (%72)	6.99	0.008
		I/II	24 (%48)	12 (%24)	6.25	0.01
	Alleles	I	70 (%70)	84 (%84)	4.78	0.03
		II	24 (%24)	12 (%12)	4.10	0.04

tution at position -31 of IL-1β may take role in the increasing amount of IL-1β and may be one of the causes of FC.

In contrast with the results obtained from the meta-analysis that was carried out by Wu et al[18], we were not able to show significant differences in allele frequencies ($\chi^2 = 0.21$, $p = 0.89$) and genotype distributions of -511 position of IL-1β ($\chi^2 = 2.33$) between the groups. Our results are consistent with the findings of the meta-analysis that was published by Kauffman et al[19].

In the present study, the frequencies of genotypes I/I and I/II of variable tandem repeat polymorphism found in IL-1 RA were different between two groups (($\chi^2 = 6.99$, $p = 0.008$ and ($\chi^2 = .25$, $p = 0.01$). Our results are consistent with the data obtained by Chou et al[12] and Serdaroglu et al[20]. IL-1 RA is antagonist of IL-1β and probably allele I is more efficient than allele II in increased gene expression of IL-1 RA. This is supported by the *in vitro* study carried out by Santilla et al[10]. They demonstrated the elevated level of IL-1β in the presence of allele two of IL-1 RA.

Conclusions

This study shows a significant association between IL-1 RA allele distribution and FC and a poor correlation of T/C substitution at the -31 position of IL-1β promoter in FC. IL-1β gene expression may be elevated due to T allele and on the other hand IL-1 RA production may be decreased due to allele two and this may lead to an insufficient antagonistic effect of IL-1 RA to IL-1β. Further studies are needed to asses the polymorphism situation and gene expression levels of these proteins.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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