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Association Of IL-6, And IL-10 Gene Snps In Childhood Febrile Seizure

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Abstract

Background: Febrile seizure are typically defined as convulsions=that-occur in children, between/6 months to 5years, who have a fever of more,,than"38 degrees Celsius, that is not associated with an intracranial' reason such as an infection, ,head .injury, or epilepsy. It is also known as the immature brain's response to fever, which is age-dependent. As a child's brain develops, there is an increase in neuronal excitability which puts the child at the risk of febrile seizures.

Aims: the aim of the present study is to find out the association of Inflammatory cytokines (IL-6, IL-10) SNP with the onset of childhood febrile seizure. **Method-** Blood samples from patients with childhood febrile seizure will be collected in a sterile condition, and the association of SNP for both IL-6 (-597) G/A, and IL-10 (-819) C/T with disease susceptibility will be studied by using allele specific PCR. **Results:** The case-control study of 40 patients with Febrile seizure and 40 control without Febrile seizure has revealed that a substantial difference in the frequency distribution of IL-6 genotypes between the patient group and the control group where ($p = 0.041$). Besides, there was no discernible variation in the frequency distribution of IL-10 genotypes and alleles. ($p > 0.05$); therefore, none of genotypes or alleles can be regarded as risk factor or protective factor. **Conclusion:** The present study has concluded that IL-6(-597) G/A, (rs:1800797) single nucleotide polymorphism (SNP) was associated with Febrile seizure susceptibility, and GG, genotype considered as, risk factor, while genotype GA act as protective factor. However, it refers that the IL-10-819C/T (rs:1800871), genes may not represent the Febrile-seizure-associated genetic risk factor.

Keywords: FSE'febrile status epilepticus, SNP'Single nucleotide polymorphisms, SCN1A' Voltage-gated sodium channel alpha subunit 1, GABA'Gamma-aminobutyric acid receptor, IL-1RA'IL-1 receptor antagonist, LE'Limbic encephalitis, COX2' Cyclooxygenase2, BBB'Blood-brain-barrier, SFS'Simple febrile seizure , CFS'Complex febrile seizure.

INTRODUCTION

Febrile seizures (FSs) are typically defined as convulsions observed in children, between the ages of 6 months and 5 years, who are experiencing a fever of more than 38 degrees Celsius, and which are not linked to an intracranial cause such as infection, head injury, or epilepsy(1). The occurrence of FSs is observed in the community, ranging between 2% and 5%, with a recognized family history. An

epilepsy diagnosis is made in a small number of individuals(2). The majority of FSs occur in children aged 12 to 18 months. It is hypothesized that the developing brain possesses a low seizure threshold during this early age and is particularly susceptible to sudden increases in body temperature. The likelihood of experiencing FSs increases when combined with specific environmental factors and genetic predisposition(3). FSs are considered a significant neurological phenomenon in children under the age of five in Western Europe and the United States, with an assumed prevalence of 2-5% among children aged 6 months to 5 years(4). While FSs can develop in any ethnic group, they appear to be more common in Asian children, with prevalence rates of 5-10% among Indian children and 6-9% among Japanese children(5). In addition to spontaneous seizures and epilepsy, induced seizures are notably common in resource-limited regions within tropical areas, largely due to the high incidence of diseases affecting the central nervous system, such as neurocysticercosis, cerebral malaria, TB, schistosomiasis, and HIV(6). Children possessing the HLA-B5 antigen have a 4.4 times higher likelihood of experiencing febrile convulsions compared to those lacking the antigen, reflecting a substantially elevated relative risk (7).

The IL-6 gene's promoter and coding regions display a variability that may influence the cytokine's secretory response and become associated with elevated serum IL6 concentrations under specific clinical conditions(8). The Interleukin-6 gene is located on chromosome 7 (p21-24) and might contribute to the risk of febrile seizure development by promoting increased IL-6 secretion, resulting in an imbalance between pro- and anti-inflammatory cytokines and ultimately leading to febrile convulsions (9). The most notable SNPs in the IL-6 gene include -174G/C (rs1800795), -597G/A (rs1800797), and -572G/C (rs1800796). Certain SNPs have been identified as impacting the transcriptional regulation of the IL-6 gene (10). While the human IL-10 gene is situated on 1q31-32, a genomic region associated with various autoimmune disorders, including FSs, IL-6 plays a critical role in the host's response to infection by inducing fever, leukocytosis, and the production of acute-phase proteins (12).

Therefore, the aim of the present study is to investigate the association between single nucleotide polymorphisms (SNPs) in the inflammatory cytokines IL-6 and IL-10 and the occurrence of childhood FSs.

Patient and Methods

A case control study was conducted on 40 patients with FSs and 40 control children whose ages ranged from 5 months to 5 years to determine the association of SNP for both IL-6 (-597) G/A(rs1800797), and IL-10-819C/T(rs1800871) with FS. The sociodermographic data are age, gender and family history of babies.

Under the supervision of a specialist pediatrician, samples were taken from Al-Zahra Maternity and Pediatric Hospital and outpatient clinics for the time period of 8 October 2022 to 5 February 2023. In these locations, cases were diagnosed by pediatricians. The control group has been selected due to their regular calcium levels, normal complete blood counts, and general health. About 2 mL blood sample was collected from each group by sterile syringe under aseptic condition; the blood was collected in

EDTA tube, then after the total DNA have been extracted, and allele specific PCR have been done, the SNP of IL-6 and IL-10 have been studied in both group

1. **Genomic DNA Extraction:** The blood samples' genomic DNA was extracted by using Frozen Blood by FavoPrep DNA kit extraction /Korea. It is done according to the instruction manual. The purity of the DNA was determined by reading 260 and 280 nm absorbance measured with a Nano-drop spectrophotometer (THERMO, USA).
2. **ARMS PCR Technique:** an amplification-refractory mutation system polymerase chain reaction technique (ARMS-PCR) assay was performed for genotyping and detection of IL-6 and IL-10 gene SNP in FS patients and control group. This method was done according to the procedure described by Qian *et al.*, (2017); Ahmed *et al.*, (2019) as in the following steps:
3. **Preparation The Primers Suspension:** The lyophilized primers were dissolved in deionized distilled water to create a stock solution with a concentration of 100 pmol/l, as directed by the manufacturer, to make the primers**,** as shown in **Table-(1**)

Table (1) Sequences of primers used for allele specific PCR.

Gene	S	P
IL-6(-597) C	**F** **F** **R**	**4**
IL-10(-819) C	**F** **F** **R**	**2**

4. **ARMS- PCR Master Mix Preparation**

An ARMS-PCR master mix was prepared by using (**GoTaq® G2 Green Master Mix kit**); it has done two reactions for each samples according to company instructions as in the following tables:

1- IL-6-597G/A (rs1800797) ARMS- PCR reaction Mix as show in table (2):

Table (2): IL-6-597G/A (rs1800797) ARMS- PCR reaction Mix

ARMS PCR Master mix	V
DNA template	8
Forward primers (10pmol)	2
Common Reverse Primer (10pmol)	2
G2 Green Master Mix	1
Total volume	2

2- IL-10-819C/T(rs1800871)ARMS- PCR reaction Mix as show in table (3):

Table (3): IL-10-819C/T(rs1800871)ARMS- PCR reaction Mix

ARMS PCR Master mix	V
DNA template	8
common Forward primer (10pmol)	2
Reverse Primers (10pmol)	2
G2 Green Master Mix	1
Total volume	2

Following that, the PCR master mix components mentioned in the table above were moved to an Exispin vortex centrifuge and centrifuged at 3000 rpm for 3minutes. The samples were then put in a PCR thermocycler (BioRad. USA).

Results

The frequency distribution of IL-6 genotypes was significantly different between the patient group and the control group.(*p* =0.041). Genotype GG was more common in the patient compared to the control group., 28 (70.0 %) versus 18 (45.0 %), respectively, thus it acts as a risk factor with an etiologic fraction of 0.40 and odds ratio of 2.85. Genotype GA was less common in the patient group than in the control group, 10 (25.0 %) versus 21 (52.5 %), respectively, thus it acts as a protective factor with a preventive fraction of 0.43 and odds ratio of 0.30,Genotype AA was equally prevalent in both the patient and control groups; hence, it cannot be a risk or protective factor. Moreover, there was no discernible variation in the frequency distribution of alleles between the patient group and the control group (p= 0.092), they cannot constitute risk or protective factors, as shown in **Table (4) below:**

Table(4): Comparison of IL-6 frequencies of genotypes and alleles in the patient group versus the control group

IL-6 G/A	**Patients group *n* = 40**	**Control group *n* = 40**	***P***	**OR**	**95 % CI**	**EF**	**PF**
Genotypes							
GG, *n* (%)	28 (70.0 %)	18 (45.0 %)	0.041 C *	2.85	1.14 -7.15	0.40	---
GA, *n* (%)	10 (25.0 %)	21 (52.5 %)		0.30	0.12 -0.78	---	0.43
AA, *n* (%)	2 (5.0 %)	1 (2.5 %)		2.05	0.18 -23.59	0.34	---
Alleles	**Patients group *n* = 80**	**Control group *n* = 80**	***P***	**OR**	**95 % CI**	**EF**	**PF**
G, *n* (%)	66 (82.5 %)	57 (71.3 %)	0.092 C NS	1.90	0.90 -4.04	0.25	---
A, *n* (%)	14 (17.5 %)	23 (28.8 %)		0.53	0.25 -1.12	---	0.25

significant at $p \leq 0.05$

Figure-1. Image of IL-6, SNP G/A, and 597 gene polymorphisms analysed by ARMS-PCR run on 2% Agarose gel electrophoresis, where stand for marker (1500- 100bp).

■ comparison the genotype and allele frequencies of IL-10 in the patient group and the control group is shown in **table-5**.Between the patient group and the control group, there was no discernible variation in the frequency of Genotype distribution and alleles.($p > 0.05$); therefore, none of genotypes or alleles can be regarded as risk factor or protective factor

Table-5: Comparison of IL-10 frequencies of genotypes and alleles in the patient group and the control group

IL-10 C/T	**Patients group *n* = 40**	**Control group *n* = 40**	***P***	**OR**	**95 % CI**	**EF**	**PF**
Genotypes							
CC, *n* (%)	2 (5.0 %)	1 (2.5 %)	0.539C NS	2.05	0.18-23.59	0.34	---
CT, *n* (%)	36 (90.0 %)	38 (95.0 %)		0.47	0.08 -2.75	---	0.35
TT, *n* (%)	2 (5.0 %)	1 (2.5 %)		2.05	0.18-23.59	0.34	---
Alleles	**Patients group *n* = 80**	**Control group *n* = 80**	***P***	**OR**	**95 % CI**	**EF**	**PF**
C, *n* (%)	40 (50.0 %)	40 (50.0 %)	1.000 C NS	1.00	0.54 -1.86	0.00	0.00
T, *n* (%)	40 (50.0 %)	40 (50.0 %)		1.00	0.54 -1.86	0.00	0.00

NS: not significant

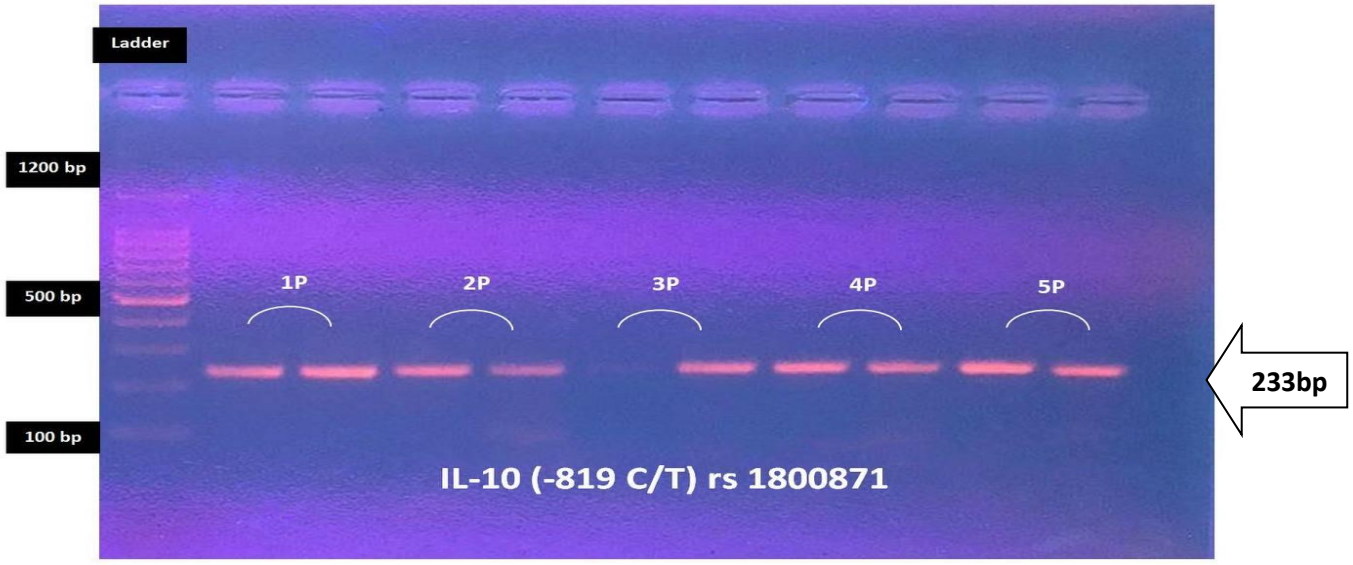

Figure 2. Image of IL-10, SNP C/T, and 819 gene polymorphisms analysed by ARMS-PCR run on 2% Agarose gel electrophoresis. Where stand for marker (1500-100bp).

Correlations of IL-10 and IL-6 genotypes to serum levels of IL-10 and IL-6 are shown in table-6. There was no significant correlation (p>0.05).

Table -6: Correlations of IL-10 and IL-6 genotypes to serum levels of IL-10 and IL-6

Characteristic	ELISA IL-10		ELISA IL-6	
	R	***P***	***R***	***P***
IL-10 C/T	-0.015	0.895 NS	0.068	0.547 NS
IL-6 G/A	-0.122	0.280 NS	-0.072	0.528 NS

NS: not significant

Discussion

The current results have shwonhthat the **IL-6-597G/A(1800797)** polymorphism may be significantly associated with susceptibility to FS. They were concordant with those of Nur et al (13) who reported that the presence of the G allele or the GG genotype at −174 and the GG genotype at −572 positions of the interleukin-6 promoter regions constituted risk factors for developing FSs in Turkish children. These findings support the hypothesis that a positive genotype predisposes individual to febrile seizure.

On the contrary, Shahrokhi et al. [14] studied IL-6 gene (−174 and +565) SNPs on genomic DNAs of 90 Iranian children with FSs, compared to 139 healthy subjects. They reported that the presence of the G allele or the GG genotype at +565 position reduced the risk of FS, while the A allele at +565 position of the promoter regions was a constituted risk factor for developing FS.

Concerning IL-10 SNP and disease susceptibility, IL-10 acts through suppression of pro-inflammatory cytokine production. Ishizaki et al reported that the frequencies of the IL-10 592C allele and 1082A/819C/592C haplotype were significantly decreased in patients with febrile seizure. Their hyperthermia-induced seizure models in immature animals showed that administration of IL-10 increased the seizure threshold temperature, and the authors concluded that IL-10 is genetically associated with

febrile seizure.(15). In previous study reported that the -592C allele and ACC haplotype in the promoter region of the *IL10* gene are significantly associated with resistance to FS. In experimental hyperthermic seizures in immature rodent models, IL-10 plays an anticonvulsant role(16).

Furthermore, the current study detected that IL-10 and IL-6 genotypes were not significantly correlated with IL-10 and IL-6 serum levels, (p>0.05), Such a result does not exclude the impact of SNP on serum level of standard cytokines**.**

Conclusion

1- The current study has concluded that there was a positive association of IL-6 SNP in patient group with febrile seizure.

2- There are also a significant difference in the distribution frequency of genotypes between the patient group and the control group, (p =0.041), and IL-6 SNP Genotype GG acts as a risk factor, while Genotype GA acts as a protective factor in FS.

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