Detection of SCN1A Gene Polymorphisms in Epilepsy Children

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Abstract: Febrile seizure is a common convulsions occur in children from 5 months to 6 years of age whose suffering from high temperature. A total of 50 children with febrile seizures and 50 normal control are included in this study. RFLP used to identify the A/G polymorphisms of the *SCN1A* gene on chromosome 2q24. Results showed that the genotype proportions and allele frequencies for *SCN1A* c.3184 A/G in both groups are not significantly. Proportions of A homozygote, A/G heterozygote, and G homozygote for *SCN1A* c.3184 A/G were as follows: in patients with FSs, 20%, 50%, and 30%, respectively compared to controls (24%, 62%, and 14%, respectively). The allele A and G frequencies for *SCN1A* c.3184 A/G in patients with FSs was 45% and 55% respectively compared to control group. The frequency of AG genotype of *SCN1A* c.3184 A/G polymorphism was non-significant in FSs compared to control group. There was increased in glucose, phosphorus levels and decrease in sodium, chloride levels. The results do not suggest that *SCN1A* p. Thr 1067 Ala or c.3184A/G (rs2298771) are susceptibility factors for febrile seizures and the fever plays an important role in causing disturbances in electrolyte balance.

Keywords: Febrile seizures, *SCN1A*, Single nucleotide polymorphisms (SNPs), PCR-RFLP .

1. Introduction

Epilepsy is a prevalent neurological disorder in children and can have a major effect on a child's development. Epilepsy begins in childhood in 60% of cases and most of the clinically significant sides of the disease occur during childhood [1]. Febrile convulsions (FC) or febrile seizures (FSs) are seizures that related with fever as high as 38.5^{o}C during childhood with an approximate rate of (3– 5)% without any infection within the central nervous system (CNS). They are the most common forms of childhood seizures. FSs are typically divided into two types, simple and complex [2]. Accumulating of epidemiological evidence indicate that FSs are the most common recognized previous for epilepsy in childhood, although the precise risk of developing epilepsy after febrile seizures is unclear. Following a first FSs, (2– 4)% of children will experiment at least one unprovoked seizure, a risk four times that in the overall of people [3] and most of these children will subsequently develop epilepsy. Factors that uniformity increase the risk for developing unprovoked seizures (epilepsy) following FSs, include a family history of epilepsy, complex features, and the existence of early onset neurodevelopmental abnormalities [4]. The incidence of FS is inconstant, ranging from (2- 5)% in North America and Europe to as much as Japan and (14)% in marina island. Data from developing countries are finite possibly because it may be very risque to differentiate simple FSs from seizures related with CNS infection. In the United States and Western Europe, they occur in (2- 4)% of all children [5]. Febrile convulsion happen more commonly in boys than in girls [6] .In fact, FSs of children include a complex interaction between the immune-inflammatory process, cytokine activation, and genetic factors [7]. Modern molecular genetic studies have disclose that mutations of the voltage gated sodium channel genes of α 1-subunit (*SCN1A*) was related with febrile convulsion. Voltage-gated sodium channel is heteromeric protein that is consists of one alpha and one or more beta subunits. The alpha subunit is responsible for channel functions and the beta-1 subunits regulate the channel kinetics. SCNA gene family consists of (9) genes (SCN1A, SCN2A, etc) and encodes the alpha subunit [8]. Single-

nucleotide polymorphisms are the most abundant types of DNA sequence variation in the human genome [9]. It is a single base pair on the DNA that varies from person to person. Single nucleotide polymorphisms are markers that may provide a new way to identify complex gene-related diseases such as FSs [10]. This study aimed to investigate the role of *SCN1A* gene polymorphism in children with febrile seizures in Al-Samawh city.

2. Materials and Methods

2.1. Patients and Blood sampling

Blood was obtained from 50 children of ages ranging from 6 months to 6 years voluntary from the Emergency Room whose visit to the Female and Children Educational Hospitals in the AL-Samawah city during the period from December 2015 to February 2016. An informed consent was obtained from the parent(s) of each child before commencement of the study. Age- and sex matched healthy children, with no history of FSs served as a control group, all these children had normal neurological examination. Diagnosis of FS and its sub classification to simple and complex FS was done according to the guidelines of the International League Against Epilepsy and divided into 2 portions: 1 ml of whole blood is collected into tubes containing EDTA (ethylene demine tetra acetic acid), kept at –20ºC, for genomic DNA extraction, and 2 ml in to gel tubes to obtained serum are separated immediately for biochemical tests. Serum concentrations of electrolytes were performed in the hospital laboratory .Glucose, creatinine and potassium were examined by Reflotron plus device (Roche/ Germany) which using parameters Reflotron test reagent strips. Calcium, phosphorus, chloride and sodium were examined by Spectrophotometer Apparatus (Cecil/ England) which based on the measurement of the intensity of radiation emitted at a wavelength characteristic for a given element. Normal value of glucose level (120- 70)mg/dl, calcium level (2.1-2.5)mmol/l, creatinine level (0.7- 1.4)mg/dl, sodium level (136- 145)mmol/l, chloride level (95-105)mmol/l, phosphorus level (1.3- 2.3)mmol/l and potassium level (3.6- 5.0)mmol/l according to the hospital reference values.

2.2. Molecular Methods

2.2.1. Conventional-PCR master mix reaction preparation

PCR master mix reaction was prepared by using (2x hot start master mix) and this master mix done according to company instructions as showing in table (1).

After that, these PCR master mix reaction components that mentioned above, placed in standard PCR tubes which containing the Quick-gDNA™ MiniPrepKit that containing all other components needed to PCR reaction such as (Hot start *T*aq DNA polymerase, standard Hot start *T*aq reaction buffer, dNTPs, tracking dye and stabilizer). Then the tube placed in micro vortex centrifuge for 1minutes, Then transferred in PCR thermocycler.

2.2.2. PCR Thermocycler Program

PCR thermocycler conditions were done by using PCR thermocycler system as the following, table (2).

2.2.3. REFLP- PCR Master Mix Preparation

Polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) master mix was prepared for detection *SCN1A* gene mutation in blood samples of febrile convulsion cases in children by using restriction endonuclease (*PvuII*) that digestion of the (336) bp PCR product of *SCN1A* gene, (Recognition sequence ($^{5'\ldots CAG\hat{}CTG\ldots 3'}_{3'\ldots GTC\hat{}GAC\ldots 5'}$) this master mix done according to company instructions as following table (3).

After that, this master mix placed in micro vortex centrifuge at 3000 rpm for 2 minutes, then transferred into incubation at 37°C for 5- 15 minute. After that, REFLP-PCR product was analyzed by agarose gel electrophoresis methods that are mention in PCR product analysis.

2.2.4. Statistical analysis

several statistical tests used to find the significant differences among the studied parameters of patients children with febrile seizures (FSs) or between the studied

parameters of patients children with febrile seizures (FSs) and control group at (P<0.05) level of significance. The biochemical tests means were compared using the least significant difference (LSD) at (P<0.05) level of significance, and the results expressed as Mean± SD. Chi-square ($\chi 2$) test was used to compare Genotypes and allele frequencies among the two groups. For all tests a probability (p) less than 0.05 was considered significant. Data processed and analyze by using statistical program social science (SPSS 22).

3. Results

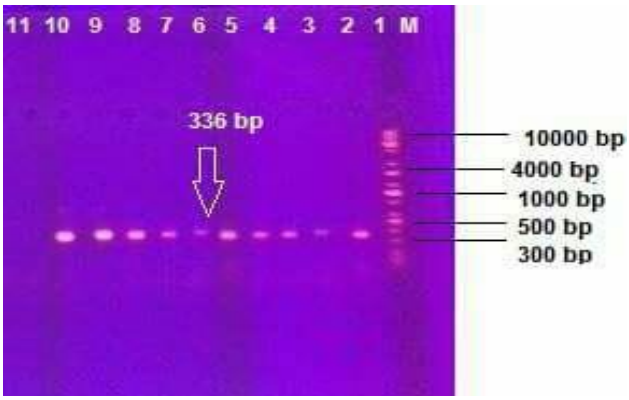
3.1. Biochemical tests

Of all 50 children with FSs and 50 children without fs served as control whose underwent to all the tests, the resulting show in table (4).

3.2. DNA Amplification

3.2.1. Voltage- gated sodium channel α-subunit type I*(SCN1A)*

The products of successful binding between the extracted DNA and specific primers for *SCN1A*gene promoter site were



detected by gel electrophoresis analysis using DNA marker and the products size was 336 bp for both patients and control groups, and the result was positive for two group, Figure (1).

Fig.(1): Ethidium Bromide-Stained Agarose Gel of PCR Amplified 336 bp of *SCN1A* gene. Lane (M):DNA molecular size marker, Lane (1-8) for FS patients, Lane (9-10) for control group.

3.2.2. Detection of SCN1A c.3184 A/G Polymorphism

Genotyping was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. After amplification, PCR products were digested using specific restriction Endonucleases. The *SCN1A* c.3184 A→G polymorphism was identified by loss of the *PvuII* restriction site AA (168 bp), AG (168 /145 bp) and GG (145 bp), However, <100 bp fragments are not seen on the gel. The PCR products were directly analyzed on 1% agarose

gel by electrophoresis, and each allele was recognized according to its size. Allelic frequencies were expressed as a percentage of the total number of alleles, Figure (2).

Fig.(2): Ethidium Bromide-Stained Agarose Gel of PCR–RFLP Amplified 336 bp of SCN1A Gene for study groups. Lane (M): DNA molecular size marker, lane (1)=undigested, Lane (2)=Homozygous AA genotype (168bp), Lane(3)=Heterozygous AG genotype (168/130bp), Lane (4)=Homozygous GG genotype (130 bp). On 1% agarose gel at 50 voltages for 10 min then at 100 voltages 45 min.

The genotype proportions and allele frequencies for *SCN1A* c.3184 A/G in both patient with FSs and control are not significantly different, table (5). The most common genotype for *SCN1A* c.3184 A/G gene in FSs was A/G heterozygote. Proportions of A homozygote, A/G heterozygote, and G homozygote for SCN1A c.3184 A/G were as follows: in patients with FSs, 20%, 50%, and 30%, respectively; and in control, 24%, 62%, and 14%, respectively, The allele A and G frequencies for SCN1A c.3184 A/G in patients with FSs was 45% and 55%, respectively; and in control 55% and 45%, respectively, table (5).The frequency of AG genotype of *SCN1A* c.3184 A/G polymorphism was non– significant in patients with FS svs healthy controls.

4. Discussion

Febrile convulsions (FCs) represent the majority of childhood seizures, and patients have a genetic predisposition to their development. The genetic susceptibility to FSs seems to involve multiple genes in most instances. In our study, there were no significant differences in potassium, calcium and creatinine levels between patients and control. Similar to our Study Afsharkhas and Tavasoli, (2014) detected that among 291 patients with FSs, there were hypokalemia, and hypocalcemia in 4 and 16 cases, respectively and there were no abnormalities in serum creatinine level but there were no significant differences between patients with simple, complex, and recurrent febrile convulsions during study in renal Function in Children with Febrile Convulsions [12]. But in sodium level there is significant lower compared with control. This results agreement with Hugen*et al.* (1995) in a prospective study of 69 children with febrile convulsions, serum sodium levels were often lower than normal (52% had levels <135mmol/l) [13]. Fever plays an important role in causing disturbances in fluid and electrolyte balance. And in phosphorus level there is significant higher, but in chloride level there is significant lower. Serum electrolyte values may be abnormal in children after a febrile seizure, but this should be manifested by physical examination and history taking [14]. And there is statistically higher significant in Serum Glucose in FS cases compare controls. It's agreement with studies by Kiviranta et al., (1995) who studied the effects of convulsion and fever on the CSF and blood glucose concentrations in four different groups of children: febrile and non-febrile children, with and without convulsions. They found the concentration of glucose in the blood was significantly higher in febrile children [15]. Their results show that hyperglycaemia in febrile convulsions is not explained just by a stress reaction, evoked by the seizure, as has been hypothesized earlier, but by the influence of increased body temperature as well.

The frequency of AG genotype of *SCN1A* c.3184 A/G polymorphism was non significantly in patients with FS s*vs*. Healthy controls. Our results agreement with study by Chou IC *et al.* (2003) as they are found that genotypes and allelic frequencies for the SCN1A gene polymorphisms in 104 Taiwanese children with FSs and 83 normal control were not significantly different [7].

Recent studies provided evidence that mutations in SCN1A represent the most frequent cause of generalized epilepsy with febrile seizures plus an autosomal-dominant epilepsy syndrome. SCN1A mutations alter channel inactivation, resulting in persistent inward sodium current. It is not known if polymorphisms in this gene involved in familial epilepsies also contribute to the pathogenesis of FSs. These suggest that the SCN1A gene might not be one of the susceptibility factors for FSs. Initiation and propagation of seizures is due to misfiring of neurons in the brain, and >300 mutations in *SCN1A* gene have thus far been identified in epilepsy and other neurological disorders [16]. Lakhen*et al.*,(2009) found an association of *SCN1A* c.3184 A/G polymorphism with overall susceptibility to epilepsy. As the causal relation between *SCN2A* 56 G→A, *SCN1A* c.3184 A→G polymorphisms and FSs associated with afebrile seizures or idiopathic generalized epilepsy has not been proven genetically, identification or confirmation of the association in different populations would be important in establishing a role for the *SCN1A* and *SCN2A* gene in the development of seizures [11].

Conclusions: Our study not find any association between *SCN1A* c.3184 A/G (rs2298771) SNPs polymorphisms in children with FSs and the fever plays an important role in causing disturbances in electrolyte balance.

Recommendation: preferably prospective studies with larger sample size are needed to clarify the role of *SCN1A* variants in the genetic susceptibility of FS as well as that of epilepsy in general, advanced molecular studies, such as gene sequencing, can be more effective in the detection of genes in FSs.

Table (1): PCR Master mix reaction and their volume.

Conventional –PCR Master mix	Volume
2x Hot start master mix	12.5µl
Forward primer (10 Pmol/µl)5′-TGCACAAAGGAGTAGCTTATG-3′	0.5 µl
Reverse primer (10 Pmol/µl)5′-AGTCAAGATCTTTCCCAATTTCAG-3′	0.5 µl
Template (0.1-1 µg for single copy)	1 µl
Nuclease free water	10.5 µl
Total volume	25 µl

Table (2): PCR Thermocycler Program for *SCN1A* [11]

PCR step	Temp.	Time	Repeat
Initial Denaturation	95°C	5 min	1
Denaturation	94°C	30 s	30 cycles
Annealing	57°C	30 s	
Extension	72°C	30 s	
Final extension	72°C	7 min	1
Hold	4 °C	Forever	-

Table (3): REFLP-PCR Master mix contents with their volume.

REFLP-PCR Master mix	Volume
PCR product	1 µl
Restriction enzyme buffer 10X	5 µl
Restriction enzyme	1 µl
Free nuclease water	43 µl
Total volume	50 µl

Table (4): The average results of chemical tests for patients with febrile seizure and control (means± standard error).

Biochemical tests	Febrile seizure (means± SE)	Control (means± SE)
Glu (120-70) mg/dl	118.28 ± 1.02 a	95.58 ± 0.79 b
$L.S.D_{0.05}$	8.444	
Crea (0.7-1.4) mg/dl	0.36 ± 0.001	0.34 ± 0.001
$L.S.D_{0.05}$	N.S	
K (3.6-5.0) mmol/l	4.55 ± 0.03	4.60 ± 0.02
$L.S.D_{0.05}$	N.S	
Ca (2.1-2.5) mmol/l	2.38 ± 0.02	2.31 ± 0.01
$L.S.D_{0.05}$	N.S	
P (1.3-2.3) mmol/l	1.80 ± 0.02 a	1.55 ± 0.01 b
$L.S.D_{0.05}$	0.177	
Cl (95-105) mmol/l	105.48 ± 0.95 b	112.76 ± 1.00 a
$L.S.D_{0.05}$	2.709	
Na (135-145) mmol/l	140.76 ± 1.25 b	147.32 ± 1.19 a
$L.S.D_{0.05}$	1.082	

[a,b]Means within columns with no common superscript differ significantly (p<0.05).N.S: non–significant.

Table (5): Genotypes and allele frequencies of *SCN1A* c.3184 A→G Polymorphisms in children with febrile seizures and normal control subjects

	Control No. (%) (n.=50)	febrile seizure No. (%) (n.=50)	P value*
Genotype AA AG GG	12 (24.00) 31(62.00) 7 (14.00)	10 (20.00) 25 (50.00) 15 (30.00)	0.0112
Allelic frequency A G	55 (55.00) 45(45.00)	45 (45.00) 55 (55.00)	0.00792
	Control No. (%) (n.=50)	febrile seizure No. (%) (n.=50)	P value*
Genotype AA AG GG	12 (24.00) 31(62.00) 7 (14.00)	10 (20.00) 25 (50.00) 15 (30.00)	0.0112
Allelic frequency A G	55 (55.00) 45(45.00)	45 (45.00) 55 (55.00)	0.00792

* P- value were calculated by X2 test .

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