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Detection of Sodium Channel *SCN1A* Gene Mutations among Patients with Epilepsy

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Abstract

Epilepsy is one of the most common neurological disorders, nearly 70% of patients with epilepsy lack an obvious pathogenetic cause, genetic is believed to play an important role in its causation. ***Objectives:*** to determine the association of sodium channel SCN1A gene mutation with epilepsy. ***Methods:*** The current study is a cross-sectional study that had been performed at Sheikh Mohamed Khair centre, Banat, Omdurman, and National Centre for Neurological Sciences (NCNS) Khartoum state, during the period November 2016 to February 2019. Ninety-nine patients were enrolled in this study. Demographic data were collected in a predesigned questionnaire. Blood samples were tested for biochemical and molecular tests. ***Results:*** sequencing analysis detected AT deletion in 71% of the samples. ***Conclusion:*** Genetic mutations have an effective role in developing epilepsy, AT deletion in SCN1A gene, indirectly, affects Gamma aminobutyric acid function which is inhibition of neuronal activity.

Keywords: Sodium Channel SCN1A, Gene Mutations, Epilepsy.

BACKGROUND

Epilepsy is a pathological state characterized by recurrent, unprovoked, epileptic seizures [1]. It affects approximately 42 million people worldwide and it is the most common heterogeneous neurological disorder with distinct symptoms, etiology, prognosis and treatments [2]. Nearly 70% of patients with epilepsy lack an obvious pathogenetic cause, genetic are believed to play an important role in its causation [3].

Among the genes known to be involved in epilepsy, the SCN1A gene represents one of the most commonly mutated human epilepsy genes, referred to be as super culprit gene [4].

The SCN1A gene codes for the alpha subunit of the neuronal voltage-gated sodium ion channel, type1 (NaV 1.1) [5], and is expressed in the central and peripheral nervous systems and in cardiac myocytes [6].

Voltage-gated sodium channels considered to be an important group of ions channels, which play an vital role in generating action potential and depolarization of the neurons **[7]**. Action potential is the electric signal that moves along the axon and sends informations to other neurons [8]. It seems logical that mutations in voltage-gated sodium channels would develop epilepsy, as these channels are in part responsible for controlling electrical excitability [9]. This study investigated scn1a mutation in patients with idiopathic epilepsy. It also determined the level of sodium, potassium.

MATERIALS AND METHODS

The current study is a cross-sectional study that had been performed at Sheikh Mohamed Khair centre, Banat, Omdurman, and National Centre for Neurological Sciences (NCNS) Khartoum state, during the period November 2016 to February 2019. 99 patients were enrolled in this study. Only the patients who were diagnosed having idiopathic epilepsy were included in this study, the rest of them were excluded.

Blood samples were drained from each patient in two containers, (EDTA) and LI -heparin, the EDTA samples were processed for DNA extraction, and the heperinized samples were used for Sodium and potassium estimations. Clinical and demographic data

were collected in predesigned questionnaire.The demographic and clinical data relating to each patient were registered (age, gender, family history, class of seizure, onset of seizure).

Biochemical analysis

Blood Serum was obtained by centrifugation technique (using Hettich Zenterfuge EBA200, Kirchlengern, Germany) at 3,000 rounds per minute for 5 minutes and then stored at -20°C. Na and K results were detected by Easylyte (Na/K full automated analyzer).

Molecular Genetic Analysis

Deoxyribonucleic acid (DNA) was extracted from whole blood via QIAGEN® DNA extraction kits (vacuum procedure). In PCR tube, 2 µl of DNA were added to 20µl of readymade MM(4 µL of 5× Firepol® Master Mix, 14 µL distilled water ,1 µL forward primer and 1 µL reverse primer) For polymerase chain reaction.

Scn1a primers used for amplification were 5' TACCCTGTTCCGAGTGATCC' 3 forward primer and 5′ GCTGTTGCCAAAGGTCTCAA3′ reverse primer, then the amplified PCR products were separated using 2% gel electrophoresis, after that , separated DNA was visualized using UV light.

DNA sequencing

Polymerase chain reaction products were transferred to china for sequencing at BGI solutions co. ltd.

DATA ANALYSIS

Statistical Analysis

Analyses was done using Microsoft Office Excel and Statistical Package for Social Science Program 2010 SPSS version 25).

Sequencing analysis

Bioinformatics tools were used to analyze sequencing results.

RESULTS

Table-1: The frequency distribution of demographic data

		Count	Column N %	P-value
Age	More than 65	2	2.0%	.000
	Less than18	32	32.0%	
	18-40	55	55.0%	
	41-65	11	11.0%	
Gender	Female	53	53.0%	.549
	Male	47	47.0%	
onset of seizure	Less than 5 yr	50	50.0%	.001
	More than 10 yr	22	22.0%	
	5-10 yr	28	28.0%	
classof seizure	Focal	4	4.0%	.000
	Focal to bilateral	26	26.0%	
	Focal with impairment	2	2.0%	
	Generalize	68	68.0%	
family history	No	90	90.0%	.000
	Yes	10	10.0%	

Table-2: Descriptive statistics (Mean±S) of biochemicals

	Mean	Standard Deviation	Normal	Comment
Na	134.82	7.95	135 – 145	Normal
K	3.65	.40	3.5 – 5	Normal

DEMOGRAPHIC RESULTS

100 subjects were recruited in this study, (47% males and 53% females) with epilepsy. Patients aged between 18-40 years old were the highest category with a 55%, followed by less than 18 years (32%), 41-65 yrs (11%) and more than 65 yrs were only 2%. Onset of seizure was less than 5 yrs were 50% of the patients, 5-10 yrs 28% and more than10 yrs were 22%. 90% of the patients have a Family history with epilepsy while the rest of them (10%) have it.

Generalize epilepsy presented in 68%, focal to bilateral in 26% and focal in 6 patients two of them(2%) developed impairment (Table 1).

Biochemical results

Serum Sodium and potassium analysis showed normal results, mean 134.82 mg/dl, 3.65 mmol/l, respectively (Table 2).

Molecular results

All the patients were tested positive for scn1a gene.

Sequencing results

The sequencing findings of SCN1A gene showed that, C>G (chr2:166848853) was detected in 57% of the samples and C>T in 14%, with splice site effect. Also AT> deletion with frame shift mutation (chr2:166848848) was appeared in 71% of the samples with splice site effect.

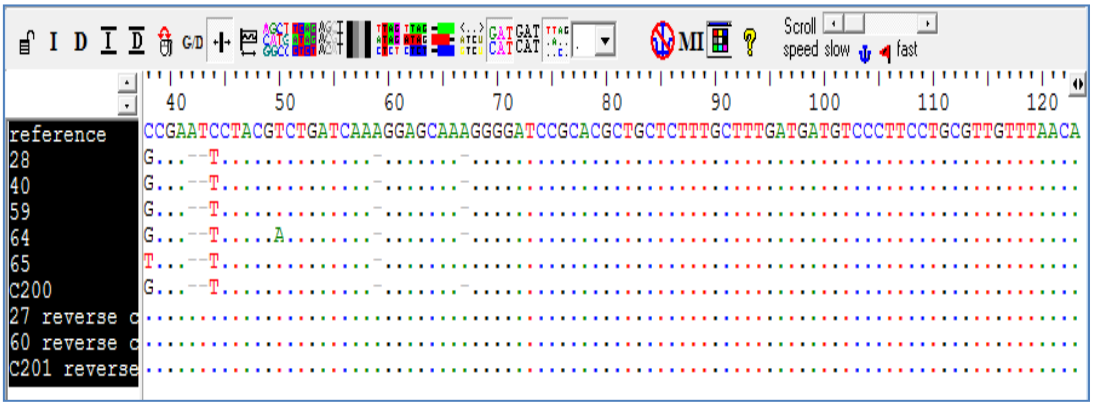

Fig-1: Show's DNA sequencing results

DISCUSSION

Epilepsy is a most common serious neurological disorder and is one of the world's most prevalent non-communicable diseases. Idiopathic (genetic) generalized epilepsies (IGEs) are common epilepsy syndromes.

Regarding onset of epilepsy, this study showed that 50% of the patients had their onset of the disease at an age less than 5 years old. Though there is increasing evidence for the existence of IGE beginning beyond the third decade [10]. Ali A. Asadi 's study only 15.2% were four years and under at the time of the onset of their disease [11].

In this study, Females outnumbered males (53% females, 47% males).similar findings were in Ali A. Asadi study (females (57%), (43%) were males.

Brain, among many other human tissues and organs, may be influenced by electrolyte disturbances. Seizures are often seen in patients with sodium disorders, especially, hypocalcemia, hyponatremia, and hypomagnesemia [12]. numerous reports suggested that the body electrolytes (sodium (na +), potassium (k +) and calcium (ca 2+) play a vital role in seizure condition to develop [13, 14], patient in the present study had sodium and potassium levels in the normal ranges this might be because samples were collected when patients were in stable status as most of them were already under treatment.

SCN1A is the most clinically relevant gene for a large spectrum of epilepsy phenotypes and the search for a mutation in the SCN1A gene is the first widely accepted step in DNA diagnosis of patients assumed to have idiopathic epilepsy.

Sequencing results showed that 71% of the sequenced samples had AT deletion chr2:166848848_166848849 resulting in amino acid substitution Isoleucine 1646 Proline. At the same exon (exon 26) Analysis in patients with GEFS+2 identified a nucleotide substitution, G4943A, that results in the amino acid substitution Arg1648His [15].

A substantial body of clinical data supports that high CNS L-proline may cause neuronal dysfunction by interfering with native neurotransmitter systems [16]. In this regard, it was noted that the chemical formation of L-proline strongly resembles that of GABA, signifying pathological levels of L-proline might specifically disrupt normal GABA-ergic function [17]. Lproline is a GABA-analogue; it causes a competitive inhibition of glutamic acid decarboxylase (GAD) by its accumulation in the cytosol of GABA-ergic neurons, leading to deficient ɣ-Aminobutyric acid (GABA) production [17]. GABA is recognized as the main inhibitory neurotransmitter in the cerebral cortex [18]. It maintains the inhibitory manner that counterbalances neuronal excitation. When there is defect in this balance, seizures may arise.

CONCLUSION

Genetic mutations have an effective role in developing epilepsy, AT deletion in scn1a gene, indirectly, affectes Gamma aminobutyric acid function which is inhibition of neuronal activity.

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