

# Cytogenetic study for Iraqi patients with epilepsy

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## Abstract

The current study aims to identify the chromosomal changes associated with epilepsy in Iraqi patients. Chromosomal analysis was carried out for 37 epilepsy patients exclusively for cases of idiopathic epilepsy (unknown cause) after excluding symptomatic epilepsy (known causes) ranging in age from 2-51 years. Only five cases showed chromosomal changes associated with epilepsy cases; their ages ranged from 2-30 years, while the rest of the patients had normal karyotypes. Chromosomes were prepared using the GTG band, with an average of 25-30 cells examined for each person.

The chromosomal changes associated with epilepsy cases ranged from several derivative chromosomes, inversion, additions, deletions, chromatid fractures, and premature centromere division (PCD). Finally, the recurrence of aberrations in some chromosomes (more than one defect associated with the same chromosome). This may indicate that these chromosomes carry more than one fragile site on the same chromosome. During the questionnaire, there were other symptoms associated with epilepsy, which have been mentioned in the study.

**Keywords:** *epilepsy, chromosomal abnormalities, Iraqi patients.*

## Introduction

According to the World Health Organization, one of the most prevalent and dangerous chronic neurological diseases affecting individuals of all ages worldwide, epilepsy is described as having a greater incidence in children and adults over 60 years of age [1].

The hallmark of epilepsy is aberrant electrical activity in the brain, resulting in seizures or strange behavior, feelings, and even loss of awareness. Prenatal or neonatal trauma, congenital brain abnormalities, head injuries, strokes, neurological infections such as meningitis, encephalitis, neurocysticercosis, and brain tumors are common causes. There may be a hereditary component to the disease in certain instances; however, this is only true in around 50% of cases. [1]

According to the WHO, 50 million people worldwide have epilepsy, 80% of them from low-income countries. [2]

There are two main types of epilepsy: idiopathic and symptomatic. In contrast to brain injury, genetic factors cause id-

idiopathic epilepsy. Physical defects in the brain are the source of symptomatic epilepsy [3,4].

Numerous chromosomal anomalies are associated with neurological changes, including central nervous system defects (CNS), which can lead to MR and more frequent seizures than the general population. [1-3]. Such chromosomal disorders as 1p36 monosomy, Wolf-Hirschhorn syndrome, ring 20 chromosome syndrome, Miller-Dieker syndrome, 18q- syndrome, and Down syndrome are uniquely linked to epilepsy and exhibit a distinct clinical and EEG pattern. In contrast to previously listed congenital defects caused by chromosomal imbalance, some congenital malformations, such as the 14r syndrome, the Klinefelter syndrome, and the Fragile X syndrome, do not exhibit particular patterns of seizures even when they occur frequently. [5].

Gene mutations are considered an important pathogenic factor for familial epilepsy syndromes and the previously thought-to-be idiopathic condition of epilepsy in individuals without a documented family history. [6].

Full trisomy 18 and the 18q deletion syndrome are two chromosomal 18 disorders commonly linked to epilepsy. Although trisomy 18 is associated with partial and generalized epilepsies beginning in the first year of life and a varied prognosis, most individuals with 18q deletion syndrome ex-

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perience focal seizures during the early years of life with a reasonable response to valproic acid or carbamazepine. [7].

The patient who had a terminal deletion of 6q suffered bouts of convulsive status, as well as callosal agenesis and oculocephalic ventricular dilatation. On CT scans, the trisomy 8q patient showed a symmetrically calcified globus pallidus and complicated partial and astatic seizures. Vomiting was often associated with episodes of gaze. Anticonvulsants well managed Down syndrome and 13qK kids who experienced infantile spasms. [8,9].

Epilepsy and chromosomal abnormalities are highly correlated. These included terminal deletions of chromosomes 1q and 1p, ring chromosomes 14 and 20, Wolf-Hirschhorn (4p-) syndrome, Miller-Dieker syndrome (del 17p13.3), Angelman syndrome (del 15q11-q13), the inversion duplication 15 syndrome, and chromosomes 15q11-q13. [10]

Once the chromosomal abnormalities increased in the last

decades, the study aimed to detect how chromosomal abnormalities may contribute to epilepsy in a sample of Iraqi patients.

## Materials and Methods

### Samples:

This cross-sectional study was conducted in 37 epilepsy patients admitted to the Al-Yarmouk Teaching Hospital. Only nine of the thirty-seven were classified as idiopathic epilepsy, while the rest were symptomatic epilepsy. The nine patients with idiopathic epilepsy underwent a chromosomal analysis. One of the patients was a 2-year-old baby with brain atrophy and seizures, and chromosomal analysis was also performed for his parents. Other patients range from 14-30 years old with other associated symptoms, as seen in Table 1.

**Table 1.** The patients age, gender and associated symptoms with disease.

Patient number	Age(years) / Gender	Symptoms associated with the disease
1	30Y ♂	Seizures, Cryptorchidism, and Morbid Obesity
2	28Y ♂	Seizures, aborted, and deformed fetuses.
3	14Y ♀	Seizures and Mental retardation
4	27Y ♀	Normal phenotype (the mother)
5(4a)	2Y ♀	Brain atrophy and seizures

### Cytogenetics.

The cytogenetic study on peripheral blood cells was performed using a short-term culture technique [11] at the Iraqi Center for Cancer and Medical Genetic Research. Briefly, stimulated peripheral blood cells with phytohemagglutinin (PHA) were cultured for 72 hours at 37 °C. Cells were exposed to colcemid (0.2 µg/ml) in the last 30 minutes of culturing time at 37 °C and were harvested, then the G-banded metaphases for chromosomal analysis were ready for karyotype designation. The International System for Human Cytogenetic Nomenclature was used [12] for the designation.

or deletion in the q arm at 1q43-44. Chromosome 16 was involved in an abnormal karyotype; it showed chromatid break at 16p13-ter and chromatid break at in16q24, as shown in figures 1,2,3.

In the second case, who had seizures, suffered from his wife's recurrent abortion and had deformed fetuses, the 30 cells revealed an abnormal karyotype with derivative chromosome 2; it could be a deletion in the short arm p25 until the terminal shown in Figure -4. Furthermore, deletion of chromosome 12 in the 12q21-23 region 12q21-23 is shown in Figure 6. Only five cells showed premature centromere division, as shown in Figure 7.

The third case, which had seizures and mental retardation, showed an abnormal karyotype; the defect included chromosomes 2 and 3. One copy of each chromosome is replaced by a derivative one (the derivative chromosome is the one that breaks and reunions randomly).

The fourth case was the mother of the fifth case. The mother had an abnormal karyotype with a deletion in chromosome 5 at q12. While the fifth case had additional abnormalities to derivative chromosome 5. It was a derivative of chromosome

## Results

Thirty-seven epilepsy patients underwent chromosomal analysis; however, only five showed chromosomal abnormalities. In the first case, who had seizures associated with other abnormalities, cryptorchidism, and morbid obesity, he showed a mosaic cell line; 14 of 25 cells analyzed were normal, while the other cells had abnormalities in chromosome 1, It was a chromatid break in chromosome 1 p36-ter

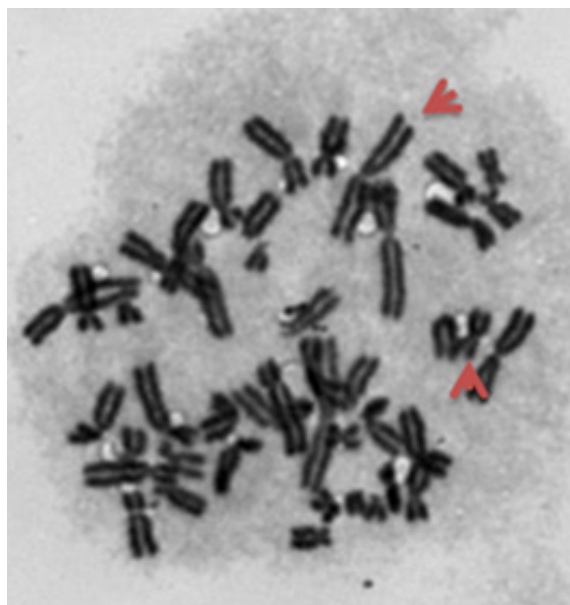
6, which could be deleted at 6q13, and there was an inversion at 6q24-21 and deletion of 6q25-27, resulting in the deriva-

tive form of chromosome 6; all the above data are presented in Table 2

**Table 2** Chromosomal abnormalities associated with patients.

Patient number	Age(years) / Gender	Chromosomal karyotype	Symptoms associated with the disease
1	30Y ♂	46, XY [14]/46, XY, chtb (1) (p36-ter), del1q43-44[3], 46, XY chtb (16) (p13.1-ter) [4]/ chtb (16) (q24) [4].	Seizures, Cryptorchidism, and Morbid Obesity
2	28Y ♂	46, XY, -2, der (2) (Del 2p25-ter?), -12, del 12q21-23[30]: PCD [5].	Seizures, aborted, and deformed fetuses.
3	14Y ♀	46, XX, -2, +der 2, -3, +der3[20].	Seizures and Mental retardation
4	27Y ♀	46, XX, -5, +der5(del 5q12).	Normal phenotype (the mother)
5(4a)	2Y ♀	46, XX, -5, +der5, -6, +der 6 Del 6q13: inv24-21: del 6 q25-27	Brain atrophy and seizures

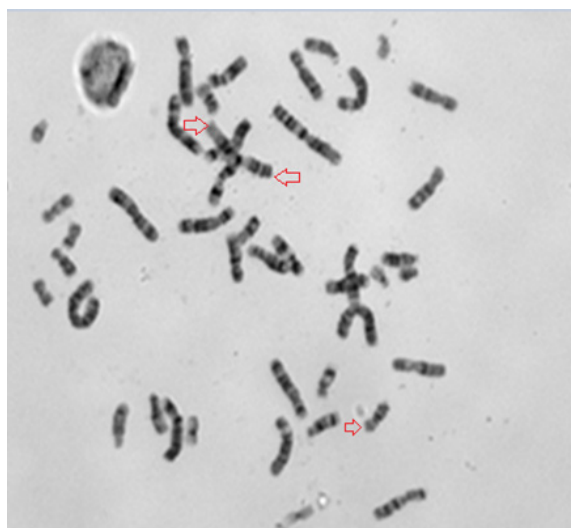
\**cthb*: chromatid break, *del*: deletion, *der*: derivative, *PCD*: premature centromere division, *inv*: inversion, and *ter*: terminal.



**Figure1-** A solid stain metaphase cell of peripheral blood lymphocyte of patient No 1 revealed: Chromatid breaks are mentioned with a red arrow, under (100X) magnification.



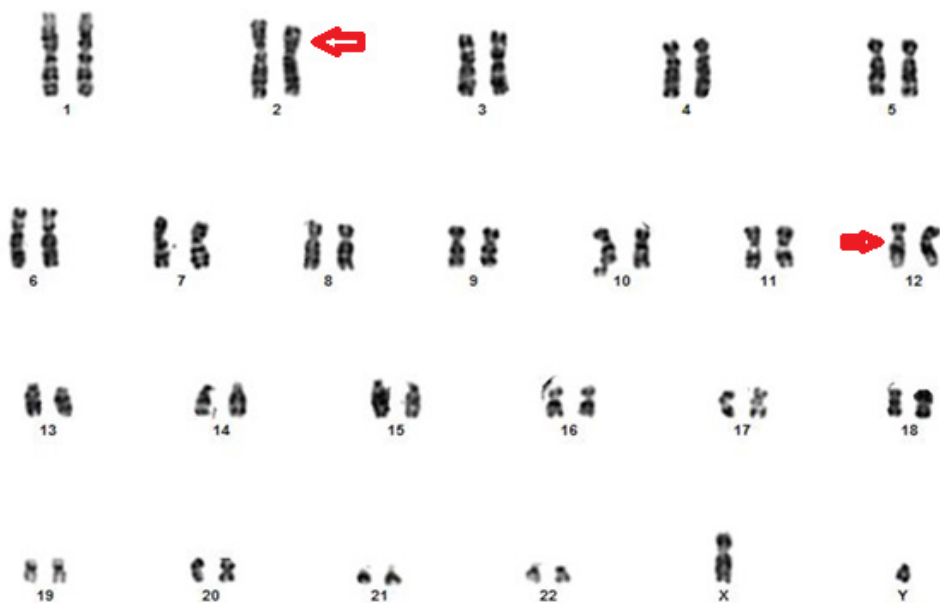
**Figure2-** G-banding metaphase cell of the G band of peripheral blood lymphocyte of patient No.1 revealed the chromatid breaks mentioned with the red arrow on chromosome 1 and chromosome 16., under (100X) magnification.



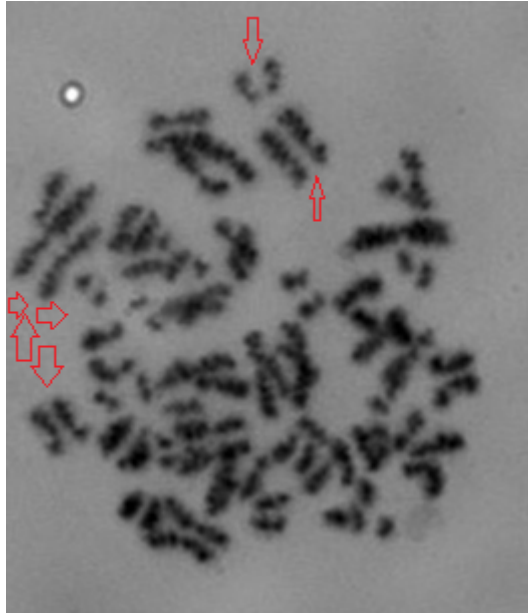
**Figure 3:** G-banding metaphase cell of peripheral blood lymphocyte of patient No.1 revealed : Deletion on chromosome 1 at 1p; 1q43-44 and deletion in chromosome 16p, under ( 100X ) magnification



**Figure 4,** a partial karyotype for the G-banding chromosome 2 at the left normal chromosome 2, at right derivative chromosome 2, under ( 100X ) magnification.



**Figure 5-** Karyotype for patient No.2 revealed one copy of the derivative chromosome 2 and one copy of the derivative chromosome 12, at (100X) magnification.



**Figure 7-** A solid stain metaphase cell of peripheral blood lymphocytes with multi premature centromere division of patient No.2 revealed: premature centromere division, at (100X) magnification.



**Figure7-** patient No. 3 revealed a translocation between chromosome 2 and chromosome 3.  $t(2,3)(q12-14,q25.3)$  a deletion of chromosome 3 translocated to chromosome 2. Under (100X) magnification.



**Figure 8,** The insertion of the translocated segment in the q part of chromosome 2 in patient No. 3 under (100X) magnification.



**Figure 9,** deletion on chromosome 5q12at right side for patient No.4.under (100X) magnification.



**Figure10-**patient No.5 revealed that one copy of chromosome 5 was derivative in addition to one copy of derivative of chromosome 6. Under (100X) magnification.

## Discussion

The first case had mosaic cell lines, one of the normal cell lines 46, XY while the other showed XY, chub (1)(p36-ter),del1q43-44[3], 46, XY chub (16)(p13.1-ter)[4]/chub (16)(q24)[4], three chromatid breaks at 1p36-ter,16p13.1-ter and 16 q24. The most frequent break region on chromosome 1 is 1p36. This region is recorded in testicular cancers [13] and is also involved in several cases of epilepsy (difficult treatment) in 58-44% of people with deletion in region 1p36, and 25% of them have problems with external progeny and the decline of cryptorchidism testicles [14]. Some cases of obesity have also been recorded as being associated with the deletion of this region. [15] Furthermore, deletion 1q43-44, recorded

in three cells of the cells studied, shares several congenital disabilities and brain defects. [16] In addition, the two other regions of 16p13.1 are recorded in cases of epilepsy[17], and region 16q24 accompanied cryptorchidism cases [18]. Therefore, it could be observed that more than one region on different chromosomes can be involved in causing a defect because genes that share a specific signal pathway for the formation and growth of a particular organ can be on different chromosomes and that post-chromosome dysfunction can disrupt the functioning of these genes and disrupt the pathway responsible for the formation of the organ.

The second patient was suffering from epilepsy and was treated with Tegretol; however, he had aborted and deformed fetuses (he had a bad obstetric history represented by repeat-



ed abortions and congenital malformed fetuses). The karyotype revealed three derivative abnormalities of chromosome 2, and thirty metaphase cells showed der2 (del 2p25-ter) with a deletion in chromosome 12 in addition to premature centromere division for five metaphase cells:

46,XY,-2,der2,-12,del 12q21-23 [30]  
+ PCD[5]

Premature centromere division PCD, a low frequency condition that can be associated with abnormalities in the brain and embryo, PCD is a significant cause of Robert Sphenocoma syndrome, which includes abnormalities of the limbs, heart, skull, and face, as well as retardation [19]. Pregnancy in the fetus can also be terminated from week 24 due to multiple fetal abnormalities caused by PCD [20]. The serious PCD makes chromosomes act during metaphase as if they were in the anaphase, affecting the cell cycle and then turning it into an abnormal cell with aneuploidy chromosomes, which explains why embryos are aborted or deformed.

There was a deletion in the 2p25-ter region in addition to changes in the long arm of chromosome 2, deletion in 2q12-21, and the region between 2q22-24 and 2q32 was duplicated(dup2q31); this piece could be duplication or a received piece from another chromosome the rearranged of chromosome 2 makes it derivative chromosome 2. Another defect was deletion 12q21-23.

The break points included in this case are the 2p25-ter, 2q22, and 12q21 regions that can break and rearrange [21]. The Weizmann Institute of Science gene cards state that the region 2p25 contains 246 genes, one of them the MYTIL gene along 2p25.3, which expresses neurons. [22] Therefore, the loss of this region explained both the epilepsy in the case and the deformed fetus. The LRP1B gene, located in 2q22.1, has a biological role in the central nervous system. On the one hand, this region plays a role in the occurrence of congenital malformations [23,24]. The second break point, 12q21, is a rare deletion point associated with several congenital disabilities, developmentally delayed problems, and heart and kidney abnormalities [25].

In the third case, who had epilepsy with mental retardation, the karyotype of 20 cells revealed had translocation between chromosome 2 and chromosome 3 at p12-14,q25 respectively:

46,XX,-2,+der 2,-3,+der3[20].

The involved region 2p12-q14 is registered in several mental retardation cases [26], and the other region 3q25 loses its activity by translocation. Loss of the region was recorded in cases with learning difficulties, autism, and other defects [27]. The translocation between chromosomes 2 and 3 did not lose genetic material but lost its effect; since both regions are involved in mental retardation, it may be a candidate for epilepsy, as we see in this study.

Case 4 and Case 5 are relatives; Case 4 is the mother who had a deletion at 5q12 and is healthy; the mother passed on the damaged chromosome to her daughter. The latter, in turn, had brain atrophy. The 5p12 region causes suppression of the

lateral branches of the neurotransmitter and has a role to play in brain development. At the same time, deletion of 5q12 affects DNA role and accumulation of damaged DNA; The ERCC8 gene expresses one of the proteins DNA repair [29], leading to cell, tissue, and then organ death, which contributes to growth failure [29]. This explains the daughter's brain atrophy that had a defect in brain tissue.

On the other hand, deletion of 5q12 could affect chromosome instability [30], and this could explain the other imbalance on chromosome 6, where deletion appeared in region 6q25-27 and inversion, 6q27 which carries many genes responsible for the production of proteins that affect brain composition, causing a malfunction in this region and loss of them, and thus causing loss of proteins that are essential for neurons and the brain. [31] it has explained the occurrence of atrophy of the brain tissues of the child (case 5). Chromosome 6 had a role associated with some rare brain atrophy [32].

### Conclusions:

This study is the first in Iraq to investigate chromosomal aberrations associated with epilepsy. Therefore, there is a limitation in the small sample size, which could not represent all chromosomal aberrations in the Iraqi population. More cytogenetic and molecular studies are needed to improve the results. This could make primary data useful for diagnostic purposes and for clinical treatment decision making.

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### Conflict of Interest:

There is no conflict of interest

### Author's Contribution:

All authors participated equally in study conception, designed data collection, interpretation of finding, manuscript, drafting and critical review.

### Ethical Approval:

The study protocol was approved by the Scientific Committee of the Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq, dated 25 January 2021. According to the first scientific committee counseling in 2021/3ed Item. Ethical statement for the study was in accordance with the ethical standards from Ethical committee of the Iraqi Ministry of Health and Environment and teaching hospital to use the specimens included in the study and the clinical information of the patients and with the Helsinki Declaration of 1975.

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