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Brain Atrophy in Iraqi Children Associated with Chromosome ٦ Abnormality

[١]Asma A. Ahmed; Nadia T. Barakat; Amal M. Ali; Noor H. Ismail; Nahi Y. Yaseen; Noor Thaer and Fatimah A. Muttlak.
Iraqi Center for Cancer and Medical Genetics Researchs, University of Almustansiriyah.
[١]E-mail: asma.amer@iccgmr.org.

Abstract

Brain atrophy is one of the congenital malformations that increased recently among neonatal children in Iraq. Brain atrophy (BA) which means a decrease in size of brain or cells number caused by different factors one of them genetic factors. Since we lack the genetic bases on such cases, the objective of the study was to shed light on the impact of genetic factors causing brain atrophy. Chromosomal analysis was used to determine the genetic affecting alterations that may involve in brain atrophy. G-banding technique was used to diagnose chromosomal abnormalities in the thirty cases and their families. The results revealed different chromosomal abnormalities such as break, translocation and inversion (rearrangements) caused BA in children while their parents seemed with normal phenotype. In addition of deletion in the short arm of chromosome ٦ which did not exceed globally ٣٠ cases until ٢٠٠٥ was recorded. This deletion had a lethal effect on the new born baby with BA. Interestingly, this study showed a relationship between chromosome ٦ and its effect on the safety of the brain.

Keywords: *Brain atrophy, Chromosome analysis, G-band.*

Introduction

It has been noted recently an increasing of various congenital anomalies that need to be diagnosed genetically to make sure of their causes in Iraq. One of these anomalies recurrences of brain atrophy. The brain atrophy BA (which means decrease of brain volume or brain cells number) which has effect on the upper and lower limbs. The serious of brain atrophy is the size that cannot be restored [١]. BA could be result from different causes such as aging, infections, autoimmunity diseases and genetic factors [١,٢]. Genetic factors include the whole genome from gene till chromosome or chromosomes. The alteration could include gain or loss of the genetic material, chromosomal abnormalities are divided to numerical or structural. Numerical abnormalities involve the loss or gain of one or more chromosomes referred as aneuploidy. While the structural chromosome abnormalities referred to the rearrangements which result from breakage with subsequent reunion in a different configuration. They can be balanced and unbalanced. The balanced one means no loss in the genetic material. Consequently balanced rearrangements are generally harmless with the except of rare cases in which one of them the break points damages an important functional gene. However carriers of balanced rearrangements are often at risk of producing children with unbalanced chromosomal complement. When a chromosome rearrangement is unbalanced the chromosomal complement contains an incorrect amount of chromosomal material and the clinical effects are usually serious [٢,٣,٤,٥,٦]. There is no studies included the impact of genetic alteration as one of the important causes of BA, this study was aimed to shed light on this spot of search .

Consequently, this study was designed to determine the effect of chromosomal changes that can be associated with the incidence of brain atrophy.

Subjects and Methods

Patients

Thirty cases were undergone for medical interviews, medical tests and magnetic resonance imaging (MRI) scans then diagnosed as brain atrophy. Then they turned to the Iraqi center for cancer and medical genetic researches (ICCMGR) for chromosomal study between ٢٠١١-٢٠١٣. The age of these cases ranged between neonatal and eleven years old children. Twenty nine of cases with BA and their parents were diagnosed for chromosomal analysis, with an exaptation of

one neonatal dead case of BA; the chromosomal study was performed for his parents. All tests were performed after obtaining the approval of the ethics committee of scientific research in the Iraqi center for cancer and medical genetic researches.

Preparation of blood specimens and cytogenetic analysis

Chromosomes were prepared from ٧٢-hour peripheral blood cells stimulated culture with phytoheamoglutnine PHA (prepared in the Iraqi Center for Cancer and Medical Genetics. Iraq). Standard procedure for cultures, harvested and slide preparation, were modified and performed in our laboratory according [٤,٧]. Briefly, ٥ ml of heparinized peripheral blood were cultured in RPMI١٤٦٠ (Sigma-Aldrich, St. Louis, MO; Schnelldrof, Germany) supplemented with ٢٠٪ fetal bovine serum (Gibco, Grand, Island, NY), and antibiotics (penicillin and streptomycin). Then, the culture exposed to١٢µg/ml Colcemid (Kreatech, Netherland) for ٣٠ minutes, followed by hypotonic treatment (KCL ٠,٠٧٥M) for ٣٠ minutes. A fixation procedure with methanol: Glacial acetic acid (٣:١) was performed freshly. Chromosomes were analyzed with GTG-banding, and karyotyping was described according to ISCN, ٢٠١٣ [٣].

Results

Only four cases were recorded for chromosomal aberrations of the ٣٠ studied cases. Ten of the cases showed normal karyotype, sixteen of the cases did not have any stimulation for growth (even their cultures were repeated for three times).

Case ١ of the chromosomal aberrations was determined in normal phenotype woman who had both copies of chromosome ٦ rearrangements, one of the copies were break in q part(long arm) and inverted, with a deletion in the ٦q١٢-١٣.while the other copy of chromosome ٦ received the additional segment which stacked to the end of ٦q(٦q+). The karyotype was ٤٦, XX, -٦, -٦,+ inv(٦) (q١٤-٢٧), + add(٦)(q,q١٢-١٣). Fig.(١, ٢).

Cases ٢ and ٣ were the two sons' of the woman, both of them received the chromosome ٦q+ copy (derivative chromosome) from their mother that means, there are partial trisomy of ٦ q ١٢-١٣ for each of them. The clinical manifestations for facial features were: squint, ptosis, depressed nasal bridge, down turned corners of mouth, micrognathia, growth deficiency especially lower limbs and optical aberration. Both karyotypes were: ٤٦, XY, -٦, + der(٦) (q, q١٢-١٣). As seen in Fig. (٣, ٤, ٥).

Fig. (١) Metaphase spread for Case ١: ٤٦, XX,- ٦, - ٦,+ inv(٦)(q ١٤- ٢٧),+add(٦) (q,q ١٢- ١٣). Magnification ١٠٠٠X.

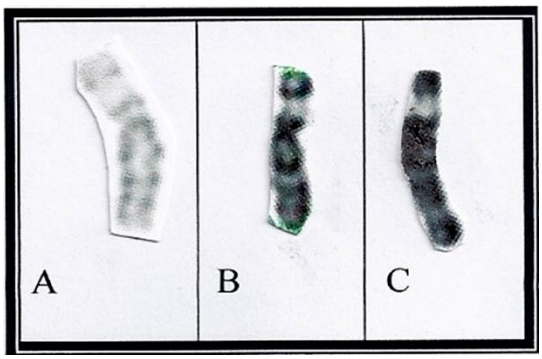


Fig. (٢) Partial karyotype A normal ٦ chromosome, B Case ١ inv (٦)(q ١٤- ٢٧) with deletion in (٦)(q ١٢- ١٣), C Case ١: add (٦)(q,q ١٢- ١٣).

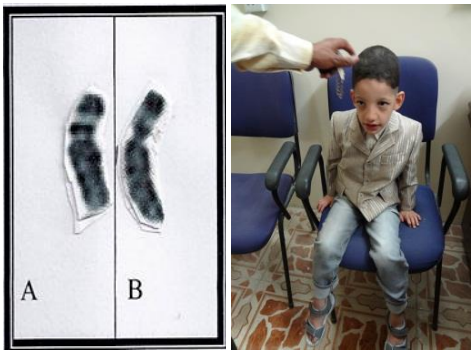


Fig. (٣) Case ٢ partial karyotype A normal chromosome ٦; B chromosome (٦)(q,q١٢-١٣); C: photo of boy with brain atrophy.

Fig. (٤) Case ٢: ٤٦,XY,-٦,+ der (٦) : add (٦)(q,q١٢-١٣). Red arrow pointed both chromosome ٦ in the spread metaphase, magnification ١٠٠٠X.

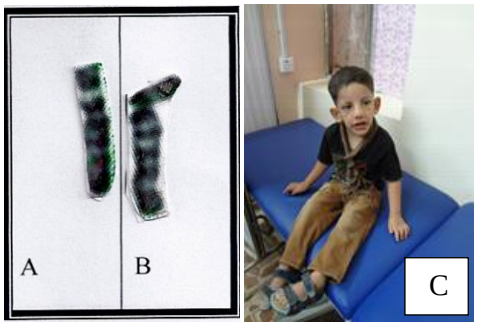
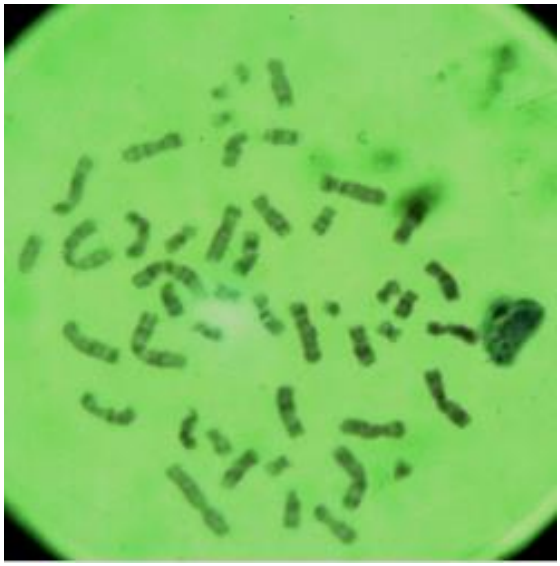


Fig. (٥) Case ٣ partial karyotype A normal chromosome ٦; B chromosome add (٦) (q,q١٢-١٣); C photo for the boy with brain atrophy.

Case ٤: newborn brain atrophy child's mother. The child dead after three days. When a chromosomal analysis was done for the parents, a defect in chromosome ٦ was been found in the mothers' karyotype. It was ٤٦, XX, -٦, + ٦del (p١١-p٢١) Fig.(٦). While mother had apparently normal phenotype.



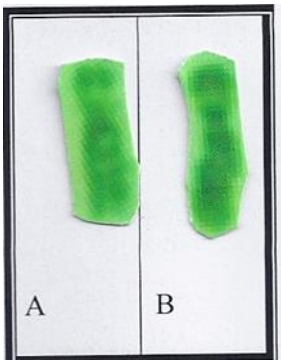


Fig.(٦) Metaphase ٤٦,XX,-٦,+del (٦)(p١١-٢١) at ١٠٠٠ X in case ٤. Partial Karyotype: A: normal chromosome ٦; Bchromosome ٦ with deletion (٦)(p١١-p٢١).

Discussion

The result of the first case was for normal phenotype mother that carries reciprocal translocation for both copies of her chromosome ٦. Her karyotype was ٤٦,XX,-٦,-٦,+inv(٦)(q١٤-٢٧),+ add(٦)(q,q١٢-١٣), which means there was no normal copy of chromosome ٦. There were two breaks on the long arm of chromosome ٦, one of them in the rejoin (٦) (q١٢) and the other one was in region (٦) (q١٤). The segment of (٦)(q١٢-١٣) left chromosome and stocked to the end of the other copy of chromosome ٦ resulting derivative (٦)(q, q١٢-١٣).While the remaining segment of long arm (q١٤-٢٧) was inverted and stoked to centromere. This break and rejoining seems that mother didn't lost genetic

material, there were just rearrangements in her chromosomes. One copy of chromosome ٦ was lack of region (٦)(q١٢-١٣) that translocate to the other copy of chromosome ٦ which had two copies of the region ٦q١٢-١٣, there was no loss of genetic material that may explains her normal phenotype[٢,٨,٩].

The second and third cases were sons who carried the derivative copy of chromosome ٦ with the addition (q١٢-١٣) (the region ٦q١٢-١٣ stocked to the ٦q٢٧) from their mother plus normal copy of chromosome ٦ from their father. It means that two copies of (٦q١٢-١٣) from their mother in one copy of chromosome ٦ and received normal copy of chromosome ٦ from their father that contain one (٦q١٢-١٣) region, so both of them have trisomy ٦q١٢-١٣.

All the above results indicate that the regions ٦q١٢, ٦q١٣, duplicated and may alert their expression in one hand and in other hand ٦q٢٧ may have been damaged while rejoining the new segment.

Other researchers recoded number of genes on those interesting regions which may have a role to affect brain development and nerve system in the children. Such as the *TFAP٢B* gene which located in the region ٦q١٢, this gene expresses protein called transcription factor AP-٢β. These proteins regulate genes that help control cell division and the self-destruction of cells are no longer needed (apoptosis). Transcription factor AP-٢β is involved in development before birth. In particular, this protein is active in the neural crest, which is a group of cells in the early embryo that give rise to many tissues and organs. Neural crest cells migrate to form portions of the nervous system, smooth muscle, glands that produce hormones (endocrine glands), pigment cells, and other tissues in the heart, and many tissues in the face and skull. Transcription factor AP-٢β also appears to play an important role in the development of the limbs [١٠].

The other duplicated region in ٦q١٣ in which the *SLC١١A٥* gene is located. *SLC١١A٥* encodes a lysosomal membrane protein called Sialin that is located mainly on the membranes of lysosomes, and play a role for sialin in the secretory processes of neuronal cells [١١]. We would like to note that lysosomes are one of the important features in brain atrophy [١]. Therefor the duplication of ٦ q ١٣ becomes a benefit trait led to brain atrophy.

The other region that exposed to the change of rearrangement is one of the important region ٦q٢٧ which include several important genes that effect in brain and CNS. The *TBP* gene the responsible gene for making a protein called the TATA box binding protein. This protein is active in cells and tissues throughout the body, where it plays an essential role in regulating the activity of most genes. It has been found that a particular mutations in *TBP* gene cause a progressive brain disorder known as Huntington disease-like ٤ (HDL٤) or spinocerebellar ataxia type ١٧ (SCA١٧). The abnormal protein builds up in nerve cells (neurons) in the brain and disrupts the normal functions of these cells. The dysfunction and eventual death of neurons in certain areas of the brain underlie the signs and symptoms of HDL٤/SCA١٧. Because the *TBP* gene is active throughout the body, it is unclear why the effects of a mutation in this gene are limited to the brain [١٢, ١٣].

Another gene that located in ٦q٢٧ is: The *T* gene which provides instructions for making a protein called brachyury. Brachyury is a member of a protein family called T-box proteins, which play critical roles during embryonic development. The brachyury protein is important for the development of the notochord, which is the precursor of the spinal column in the embryo. The notochord disappears before birth, but in a small percentage of individuals, some of its cells remain in the base of the skull or in the spine. The notochord helps control the development of the neural tube, which is a layer of cells that ultimately develops into the brain and spinal cord [١٤].

Parkin is normally abundant in the brain is the product of *PARK٢* gene which is located in ٦q٢٧, its loss could lead to the impairment or death of nerve cells, including those that produce dopamine. Loss of dopamine-producing nerve cells is a characteristic feature of Parkinson disease [١٥].

All these rearrangements may explain the brain atrophy and nerve system disorders, especially when we know that ٦٪ of human genome is located on chromosome ٦[١٦], and these critical genes that located on

chromosome ٦ have a role in controlling the development of the brain and nervous system as mentioned previously in [١٠, ١١, ١٢, ١٣, ١٤, ١٥].

Deletions in chromosome ٦ are relatively rare fewer than ١٠٠ cases reported for deletions in long arm [١٧] ,and around ٣٠ people who have deletions in short arm of ٦ chromosome with disorder have been described in published medical research [١٧,١٨]. There is very little information on people with deletions between centromere and band ٦p٢٢,١. In our study we have been recognized an interstitial deletion (٦)(p١١-٢١) in woman with normal phenotype who had neonatal dead baby after ٣ days of born with brain atrophy and other congenital malformation. The loss of DNA segment spans the region contain number of genes. It may have an impact in causing the status of the fetus' brain atrophy. Number of gene located in this region have recoded to have effect not only on brain but on other organs. Such as *RAB*٢٣ gene which located in ٦p١١ that provides instructions for making a protein, the Rab٢٣ protein which regulates a specific developmental pathway called the hedgehog signaling pathway that is critical in proliferation of the cell, cell specialization, and the normal shaping (patterning) of many parts of the body during embryonic development. one of the nonsense mutations of Rab ٢٣ in open brain mice cause recessive embryonic lethality with neural-tube defects, suggesting the importance of *RAB*٢٣ during early development [١٩].

Other genes located in ٦ p ١٢ like the *PKHD*١ gene express for a protein called fibrocystin (sometimes known as polyductin). This protein is present in fetal and adult kidney cells, and is also present at low levels in the liver and pancreas.The region contain other gene affected in other organs [٢٠]. It seems that the deletion eliminates the job of such genes located in ٦p١١-١٢ and that may be responsible for lethal effect which affected different organs not only the brain.

Forty percentage of the cases did not show any metaphases. This result needs further work to investigate the failure of obtaining metaphase in spite of repeating culturing. However this phenomena usually seen in the field of cytogenetic [٧]. May the causes prevent the stimulation of culture the same those led to brain atrophy or may brain atrophy affected their culture stimulation somehow.

This study concludes that the brain atrophy could result from different reasons that related to direct or indirect genetic factors and when associated with other disorder in the body. This study recorded another case among the rare cases in the world, is for deletion in the short arm of chromosome ٦, which did not exceed thirty-case limit in ٢٠٠٥ globally. In addition to what was confirmed by the study of the important role of chromosome ٦ in the subscription, in some cases of brain atrophy. Especially when the appearance of children with genetically defect brain atrophy from normal phenotypic parents may be because of exposed those parents to environmental conditions that changed their genotype, and they had been inherited to their children.

References

[١] Rubin R, Strayer DS, Rubin's Pathology Clinic pathologic Foundations of Medicine. ٥th ed, pp٣-٤, ٢٠٠٨.

[٢] Turnpenny PD and Ellard S. Emery's Elements of Medical Genetics, ١٣th ed. Churchill Livingstone Elsevier, ٢٠٠٨.

[٣] ISCN (٢٠١٣): An international System for Human Cytogenetic Nomenclature. L.S. Shaffer, J. Mc Gowan-Jordan, M. Schmid (eds); S. Kargar, Basel.

[٤] Therman E, Susman, M, Human Chromosome: structure, behavior and effects, ٣rdedn.Springer, New York, (١٩٩٣).

[٥] Wang JC, "Autosomal Aneuploidy", The Principles of Clinical Cytogenetics. Second Edition, Humana Press Inc. pp ١٣٣-١٦٥, ٢٠٠٥.

[٦] Mc Fadden DE and Friedman JM, "Chromosomal abnormalities in human beings", Mutation Research, ٣٦, pp١٢٩-١٤٠, ١٩٩٧.

[٧] Yaseen NY, Humadi AA, Tawfiq MS and Estivan AG, "Cytogenetic studies on patients with chronic Myelocytic leukemia", Med. J. Tikrit Univ, ٤ pp ٥-٩, (١٩٩٨).

[٨] Clark CE, Cowell HR, Telfer MA, Casey PA, "Trisomy ٦ q ٢٥ leads to ٦ qter in two sisters resulting from maternal ٦;١١

translocation", Am J Med Genet., ٥, pp ١٧١–١٧٨, ١٩٨٠.
[٩] Chase TR, Jalal SM, Martsolf JT, Wasdahl WA, "Duplication ٦ q ٢٤ leads to ٦ qter in an infant from a balanced paternal translocation", Am J Med Genet, ١٤, pp ٣٤٧–٣٥١, ١٩٨٣.
[١٠] Satoda M, Zhao F, Diaz GA, Burn J, Goodship J, Davidson HR, Pierpont ME, Gelb BD, "Mutations in TFAP٢B cause Char syndrome, a familial form of patent ductus arteriosus. Journal Nat Genet, ٢٥, pp ٤٢-٦, ٢٠٠٠.
[١١] Aula N, Kopra O, Jalanko A, Peltonen L, "Sialin expression in the CNS implicates extralysosomal function in neurons", Journal Neurobiol Dis.,١٥, pp٢٥١-٦١, ٢٠٠٤.
[١٢] Van Roon-Moma WMC, Reidb S J, Faulla RLM, Snellb, RG, "TATA-binding protein in neurodegenerative disease", Neuroscience, ١٣٣, pp ٨٦٣–٨٧٢, ٢٠٠٥. Review
[١٣] Rasmussen A, De BiaseI, Fragoso-Benítez M, Macías-Flores M, Yescas P, Ochoa A, Ashizawa T, Alonso M, Bidichandani S, "Anticipation and intergenerational repeat instability in spinocerebellar ataxia type ١٧", Annals of Neurology, ٦١, pp ٦٠٧–٦١٠, ٢٠٠٧.
[١٤] Vujovic S, Henderson S, Presneau N, Odell E, Jacques TS, Tirabosco R, Boshoff C, Flanagan AM ," Brachyury, a crucial regulator of notochordal development, is a novel biomarker for chordomas" Journal J Pathol, ٢٠٩,pp ١٥٧-٦٥, ٢٠٠٦.
[١٥] Huang Y, Cheung L, Rowe D, Halliday G, "Genetic contributions to Parkinson's disease", Journal Brain Res Brain Res Rev, ٤٦, pp٤٤-٧٠, ٢٠٠٤.
[١٦] Mungall AJ *et al*, "The DNA sequence and analysis of human chromosome ٦", Nature, ٤٢٥, pp٨٠٥-١١, ٢٠٠٣.
[١٧] Klein OD, Cotter PD, Moore MW, Zanko A, Gilats M, Epstein CJ, Rauen KA, "Interstitial deletions of chromosome ٦q gentotype-phenotype correlation utilizing array CGH", Clin Genet, ٧١, pp٢٦٠-٢٦٦, ٢٠٠٧.
[١٨] Celestino-Soper P, Skinner C, Schroer R, Eng P, *et al*, "Deletions in chromosome ٦p٢٢,٣-p٢٤.٣, including ATXN١, are associated with developmental delay and autism spectrum disorders", Mol Cytogene, ٥, pp١٧-٣٠, ٢٠١٢.
[١٩] Jenkins D, Seelow D, Jehee FS, Perlyn CA, Alonso LG, Bueno DF, Donnai D, Josifova D, Mathijssen IM, Morton JE, Orstavik KH, Sweeney E, Wall SA, Marsh JL, Nurnberg P, Passos-Bueno MR, Wilkie AO, "RAB٢٣ mutations in Carpenter syndrome imply an unexpected role for hedgehog signaling in cranial-suture development and obesity" Journal Am J Hum Genet ,٨٠, pp١١٦٢-٧٠, ٢٠٠٧.
[٢٠] Wilson PD,"Polycystic Kidney Disease" N Engl J Med, ٣٥٠, pp١٥١-١٦٤, ٢٠٠٤.

الخلاصة

ضمور الدماغ من التشوهات الخلقية التي زادت في الآونة الأخيرة بين الأطفال حديثي الولادة في العراق. ضمور الدماغ يعني اما تقليل حجم الدماغ أو تقليل عدد خلايا الدماغ والتي يمكن أن تنتج من عدة اسباب مختلفة، واحد هذه الاسباب هي العوامل الوراثية. ولما كنا نفتقر إلى الدراسات الوراثية على مثل هذه الحالات، جاء هدف الدراسة لتسليط الضوء على تأثير العوامل الوراثية أن تسبب ضمور الدماغ. وتم استخدام تحليل الكروموسومات لتحديد آثار االتغيرات الوراثية التي قد تشترك في التسبب بضمور الدماغ. تم استخدام تقنية التحزيم– G لتشخيص الشذوذ الكروموسومي للحالات الثلاثين المدروسة ووذويهم. وجاءت النتائج التي كشفت عنها الدراسة والتي تراوحت بين كسر و انتقالات جديدة من ناحية وناحية أخرى كان هناك اقلاب اي الناتج اعادة الترتيب للكروموسومات. هؤلاء الآباء الذين كانوا يحملون النمط الظاهري الطبيعي على الرغم من حملهم النمط النووي معاد الترتيب، كانوا قد نقلوا الى ذريتهم النمط المعاد الترتيب والذي نتج عنه ضمور الدماغ الذي يعانون منه. كما وسجلت في هذه الدراسة واحدة من الحالات النادرة لحذف في الذراع القصير في كروموسوم ٦ والتي لم تتجاوز ٣٠ حالة على مستوى العالم حتى عام ٢٠٠٥. كان هذا الحذف تأثير قاتل على المولود الجديد الذي كان يعاني اصلا ضمور في الدماغ. ومن المثير للاهتمام أظهرت هذه الدراسة وجود علاقة بين الكروموسوم ٦ وتأثيره على سلامة الدماغ.