Down Syndrome Mosaism in Samples of Iraqi Patients
متلازمة داون الموزائيكية في مرضى عراقيين

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Abstract:

The Down syndrome (DS) is the well-known trisomy, which is caused by additional copy of chromosome 21.There are three types of DS. First, fully trisomy (47,XY,+21or 47,XX,+21). Second, translocation DS which result as translocation between chromosome 14 and 21(46,XY,trans (14:21) or (46,XX,trans (14:21)). And the third type the mosaic DS that two cell lines present in the individual. Mostly, studies indicate that frequency of each type 95%, 4% and 1% respectively. Our study aims to estimate the frequency of each of the three types of DS chromosomal abnormalities in Iraqi samples. Chromosomal analysis using G-band technique was performed for 200 Down syndrome cases and 168 of their parents (whenever there were mosaic DS cases their parents were submitted chromosomal analysis). Fifty-seven percentage of cases were fully Trisomy, forty-three percentage of them were mosaic DS, and no translocation pattern was recorded. The maternal ages were between 25-45 for the mosaic DS mothers. According to this study, the frequency of mosaic DS was varied. It seems more investigations need to be done for larger number of DS, and the impact of environmental changes in last decades need to be studied more to be sure of its role in increasing of the proportion of this type of Down syndrome.

Key words: Down syndrome, Mosaism, Chromosomal analysis.

الملخص

متلازمة داون هي اكثر انواع متلازمات التثلث الكروموسومي Trisomy المعروفة, تنتج متلازمة داون من وجود نسخة اضافية من كروموسوم 21. هناك ثلاثة انماط من متلازمة داون اعتمادا على نوع الهيئة الكروموسومية, الاول هيئة كرومسومية ذات 47 كروموسوما اي باضافة نسخة من كروموسوم 21 في جميع خلايا الجسم وتكون بالشكل التالي: 47,XY,+21 للذكور, 47,XX,+21 للاناث. النمط الثاني ان تكون الهيئة الكروموسومية حاوية على نسخة اضافية من كروموسوم 21 منتقلة على كروموسوم 14 وبهذا تكون الهيئة 46,XY,+21trans (14,21) للذكور, و46,XX,+21 trans (14,21) للاناث. اما النمط الثالث فيكون بشكل يحوي فيه الجسم خطين من الخلايا او هينتين كروموسوميتين في نفس الشخص 46,XY:47,XY,+21 او 46,XX:47,XX,+21 . ان تكراروجود هذه الانماط الثلاثة على مستوى العالم هو مايلي:التثلث الكروموسومي الكامل لجميع خلايا الجسم يمثل 95 % بينما يشكل النمط الثاني الذي تكون الهيئة الكروموسومية حاوية على النسخة الاضافية المنتقلة لكروموسوم 14 تشكل 5 %. اما الهيئة الكرموسومية المبرقشة والتي تضم خطين خلايا 46,XY:47,XY,+21 فتمثل 1%.تهدف الدراسة الحالية تسجيل مقدار تكرار هذه الانماط الثلاثة في العينة المدروسة وهي 200 حالة من متلازمة داون و168 من ذوي الاطفال الذين يحملون هيئة كروموسومية موزائيكية (من ثبت انه يحمل هيئة كروموسومية مبرقشة تم اجراء التحليل الكروموسومي لذويه). حيث جاءت النتائج ان نسبة سبعة وخمسون بالمئة من الحالات المدروسة كانت متلازمة داون كاملة التثلث الكروموسومي فيما كانت نسبة ثلاثة واربعون بالمئة من الحالات المدروسة تحمل هيئة كروموسومية موزائيكية (اي خطين من الخلايا). من خلال العمل تم تسجيل اعمار الامهات ذوات متلازمة داون الموزائيكية والتي تراوحت ما بين 25-45 عاما. وجاءت نسبة الذكور في متلازمة داون الموزائيكية 57% فيما كانت نسبة الاناث 43%. ولم تسجل اي حالة متلازمة داون الحاوية على الانتقالات مابين كروموسوم 14 وكروموسوم 21ضمن الدراسة. وبحسب نتائج هذه الدراسة هناك تغاير في نسب تكرار الانماط الثلاثة ولذا سيكون هناك حاجة لدراسات اكثر اتساعا لحالات متلازمة داون ومقارنتها مع التاثير البيني الحاصل في العقود الماضية لتوثيق تاثير هذه التغيرات مع زيادة نسبة هذا النمط من المتلازمة.

الكلمات المفتاحية: متلازمة داون، الموزائيكية، التحليل الكروموسومي

Introduction:

Genetic disorders have a net gain or loss of DNA or alteration of the DNA sequence, which effect protein synthesis (functional product of genes). The genetic disorders account for approximately 30-45% of birth defects and may account for 50% of birth defects with unknown cause. Genetic disorders include chromosomal abnormalities, single disorders, nontraditional inheritance (non Mendelian), and multi factorial inheritance.

Chromosomal abnormalities could be either numerical or structural, are visible with the light microscope, which generally result in clinical syndromes.

Large segment of DNA and potentially multiple genes are affected because of portions of chromosomes, entire chromosomes, or the entire karyo type may be altered. Overall chromosome abnormalities occur in 5-7 per 1000 live births, 2% of pregnancies among women age over 35and older, and are responsible for 50 to 70% spontaneous abortions that occur in the first trimester of pregnancy[1,2]. Numerical abnormalities of chromosomes occur when there is gain or loss of chromosomes number that what is known as aneuploidy.

Down syndrome is the most well-known aneuploidy disorder referred to trisomy 21(gain addition copy of chromosome 21).Which isoccur1 in 700 live births. The Down syndrome's karyo type is either 47,XX,+21 for female, or 47,XY,+21 for male demonstrating a gain of chromosome 21[3,4].

A disorder is characterized as a syndrome when two or more clinically identifiable symptoms are present in the phenotype. The phenotype for Down syndrome includes short stature, the eyes may be close set with narrow slanting eyelids, short fingers, mental retardation, craniofacial features such as flat face and protruding tongue and hearing loss[5].

Chromosomal abnormalities in Down syndrome frequent 95% trisomy 21, 4% translocation between14, 21(which means there were 46 chromosome and there was brake and reunion between chromosome 14 and chromosome 21), and 1% mosaic cell lines (mosaism refer to the presence of genetically distinct groups of cells within somatic and germ line tissues, respectively [5,6]. Since there is a significant increase in environmental toxic levels in Iraq during the past decades, which is believed to affect the genetic material [7,8, 9]. The aim of this study was to estimate the frequency of each and every one of the three types of DS chromosomal abnormalities.

Material and Methods

Patients

All of tests were performed after obtaining the approval of the ethics committee of scientific research in Iraqi center for cancer and medical cancer research for the study samples. Then cases and their families were undergone for chromosomal analysis after they were originally a medical interview with them. From different regions in Iraq, and between 2002 and 2013, two hundred DS cases aged between 1 day to 56 years underwent physical examination, interviewed to medical history and underwent chromosomal analysis. After determination the mosaism, 84 couples of parents (168 male and female) were underwent chromosomal analysis too.

Preparation of blood specimens and cytogenetic analysis

Chromosomes were prepared from 72-hour peripheral blood cells stimulated culture with phytoheamoglutnine PHA (prepared in Iraqi Center for Cancer and Medical Genetics. Iraq). Standard procedures for cultures, harvests and slide preparation, were modified and performed in our laboratory according [10,11]. Briefly, the peripheral blood cells were grown in RPMI1460 (Sigma- Aldrich, St. Louis, MO; Schnelldrof, Germany) supplemented with 20% fetal bovine serum (Gibco , Grand, Island ,NY), and antibiotics (penicillin and streptomycin).Then, the culture exposed to12µg/ml Colcemid (Kreatech , Netherland) for 30 minutes, followed by hypotonic treatment KCL(0.075M) for 30 minutes. A fixation procedure with methanol: Glacial acetic acid (3:1) was performed. Chromosomal were analyzed with G-banding, and karyotyping was described according to ISCN, 2013 [12].

Result

Interestingly, a marked increase in the proportion of cases of mosaic Down that were studied compared with results globally recorded. While globally mosaic DS estimated 1%, our study estimated 43% of the DS cases were mosaic. Two hundred patients with DS were studied; 86 of them were mosaic Down syndrome. Two of mosaic DS patients were aged 54 and 56their karyo types were:

46, XY[80%];47,XY,+21[20%]and46,XY[85%]; 47,XY;+21[15%] respectively.

The mosaic DS were 86 cases (43% of study cases).Thirty –one percentages of the 43% mosaic DS cases mothers aged 20-35. Twelve percentage of mothers aged 35-45 and 0% 45 older. Thirty –one percentage of 20-35 mothers (one of the parents worked in military position, chemical work, radiation rays, fuel station workers or mothers get antibiotics during pregnancy, Thyroid drugs(thyroxin), Toxoplasmosis, CMV infections and some unknown causes). Fifty-seven percentages of the DS cases

were fully trisomy+21. No translocation DS were recorded by chromosomal analysis. The female DS were 43% of cases while the male 57%. The incidence of mosaic DS in male were57% while 43% in female.

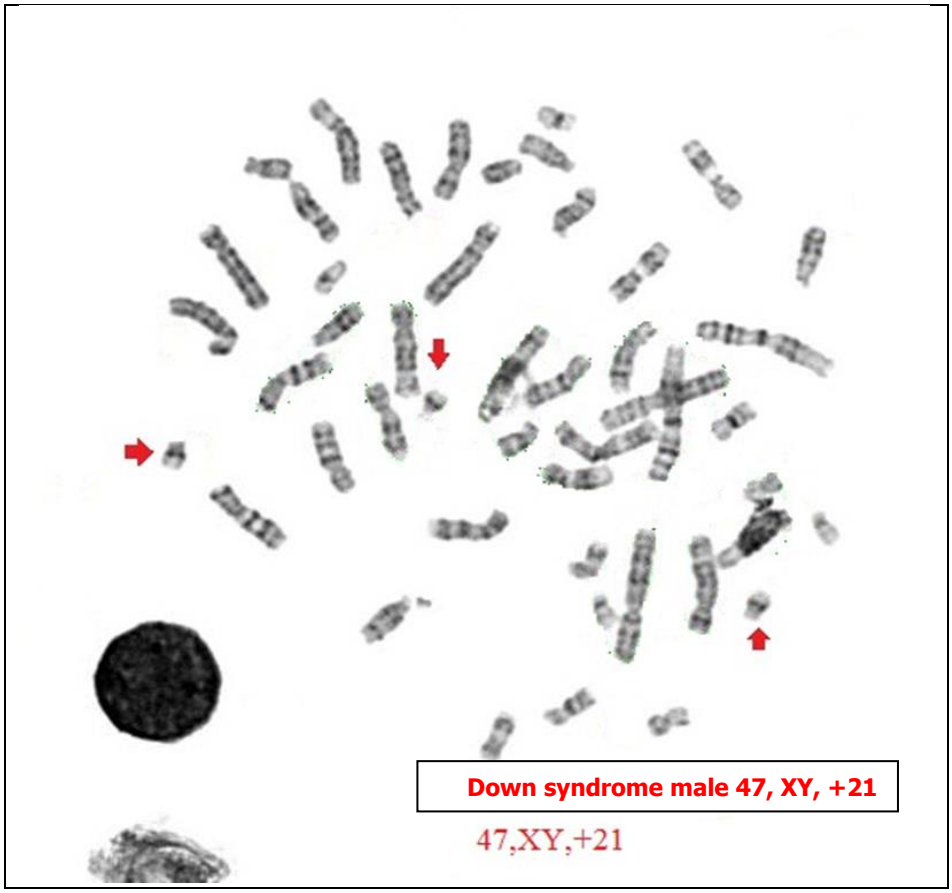

Fig. (1a): Ametaphase spread showed Downs' syndrome male:47,XY,+21.Red arrows on chromosome 21.magnified (1000)X.

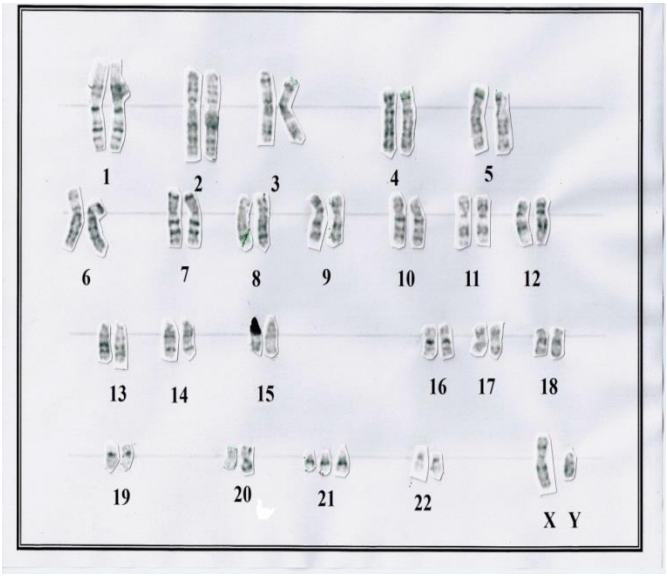

Fig. (1b): karyotype of male Down syndrome 47,XY,+21.

Fig.(2): one year's old girl Down syndrome.

Dissuasion

The trisomy occurs when chromosomes do not separate properly during meiosis and resulting gamete contain the incorrect number of chromosomes, one of them gain additional copy of chromosome while the one loss copy of the chromosome. This situation is termed non disjunction. When the gamete (ovum or sperm) which formed by no disjunction contributes to fertilization the resulting zygote. Sometimes parental dispermyenter the ovum during fertilization resulting in a zygote with additional set of parental chromosomes and overall) 3n total chromosomes [13].On the other hand, in females, the total number of eggs is present in the newborn female, and all eggs are arrested at an early stage of the first meiotic division as a primary oocyte (primordial follicle). Complete stimulation of meiosis I became after puberty, during each menstrual cycle. During this period, with advancement of maternal age a lot of causes, chemicals, stress, radiation rays, drugs, alcoholics, smoking and other factors may have effect on eggs. The all above factors may be explain the presence of the 57% of studied cases were totally trisomy 21, especially the recorded parents cases were actually exposed to these factors according to what has been registered in medical interview.

Mosaism refers to the presence of a genetically distinct cell population within an organism [14]. If the mosaism occurs only in a somatic cell population, the phenotypic effect will depend on the extent of the mosaic cell population; however, there would be no risk of passing on the mosaic genotype to offspring. On the other hand, if the mosaism occurs only in a germ line cell population, the individual would be unaffected, but his or her offspring could be affected. It may be a consequence of chromosome misalignment on a disorganized spindle or non-functional metaphase/ anaphase checkpoint point [15].These situations may explain the 43% DS mosaism in our study, Especially as recorded in the medical interview that one of parents was exposed to one of the factors: one of the parents worked in military position, chemical work, radiation ray , fuel station workers or mothers get antibiotics during pregnancy, Thyroid drugs(thyroxin), Toxoplasmosis, CMV infections. Thus, this explains the young maternal age 25-35 and the responsibility of the father in a DSmosaism. Two of mosaic DS patients were aged 54 and 56years, their karyotypes were 46, XY[80%]; 47,XY,+21[20%] and46,XY[85%]; 47,XY;+21[15%] respectively. the low proportion of trisomy mosaism may decrease the accompanying symptoms of the syndrome may allowed the patient to reach these age.

For unknown reason, the incidence of mosaic DS in male were 57% while 43% in female. No translocation type (14, 21) was estimated. According to this study, the frequency of mosaic DS was varied. It seems more investigations need to be done for larger number of DS, and the impact of environmental changes in last decades need to be studied more to be sure of its role in increasing of the proportion of this type of Down syndrome.

Finally the conclusion of this study, there was variation in DS types frequency. And increasing of mosaic type frequency may result of the environmental factors effects(which include type of work or type treatment or even type of food habits) that may overlap in the distribution of gametes leading to non-disjunction that increase mosaism proportion. Thus there is need to have more investigation studies for DS frequency on larger number with the role of environmental factors effect.

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