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Original Article

Cytogenetic findings in patients with intellectual disability and/or multiple congenital anomalies

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Abstract

Introduction: Chromosomal abnormalities are a major etiology of intellectual disability (ID) and multiple congenital anomalies (MCAs). Screening for chromosomal aberrations by clinical diagnostic techniques has been primarily performed through standard karyotyping.

Methods: A cytogenetic study involving 1730 individuals with ID and/or MCA was conducted using lymphocyte culture and high-resolution G-banding method.

Results: Various types of chromosomal abnormalities were detected in 440 patients (25.5%). Numerical and structural chromosomal abnormalities, respectively, were observed in 63.3% (278 out of 440) and 36.7% (162 out of 440) patients. Among the chromosomal abnormalities, sexual chromosomal abnormalities were found in some cases, and Klinefelter syndrome with 2.5% frequency was the most frequent sex chromosomal abnormality. Autosomal abnormalities were found in cases, and Down syndrome was the most frequent autosomal chromosomal abnormality occurring in 41.0% of detected abnormalities.

Conclusion: The higher contribution of chromosome aberrations in the northwest of Iran indicates the importance of cytogenetic evaluation in the etiology of MCA and/or ID patients. Genetic counseling was provided to the family members to explain the recurrence risk as well as the need for prenatal diagnosis in subsequent pregnancies and management of patients by collected data bank.

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Introduction

A chromosomal disorder occurs when there is a change in the number or structure of the chromosomes. This change in the amount or arrangement of, the genetic information in the cells may result in problems in growth, development and/or functioning of the body systems. The chromosomal abnormalities may occur either during the production of the egg or sperm or early after the baby's conception: a spontaneous occurrence for unknown reasons.[1]

Intellectual disability (ID) is defined as "a disability characterized by significant limitation both in intellectual functioning and in adaptive behavior as expressed in conceptual, social, and practical adaptive skills."[1] The diagnosis of ID is often primarily performed based on clinical manifestations. The heterogeneous nature of ID and/or multiple congenital anomalies (MCA), with the wide-ranging profile of the underlying etiology, is a particular challenge for the clinicians and the affected families. Characterization of the specific etiology can provide useful clinical information including prognosis, recurrence risk, and available therapy.[2,3]

Approximately, one-third of etiologic diagnoses are characterized through

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examination and history. Mendelian disorders, chromosomal anomalies or environmental factors can individually or in combination cause ID and/or MCA. Cytogenetic abnormalities, which are detected in approximately 10.0% of patients with ID and/or MCA, are a major etiology and it is difficult to give an exact estimation of their contribution to ID and/or MCA, because of the variability of parameters used in the screening processes. Chromosome analysis is an important process in the diagnosis and evaluation of genetic disorders. Identification of chromosome anomalies may clarify the cause of ID and/or MCA syndromes as well as facilitate in genetic counseling.[4] To date, around 1000 chromosomal syndromes that cause a major impact to human morbidity and mortality has been reported. The frequency of chromosomal anomalies is quite different in the various developmental stages, causing 50-60% of fetal death during the first trimester of pregnancy and 1.0% among live-born children. Major autosomal and sexual chromosomal anomalies often cause a number of phenotypic features such as ID, cardiac anomalies, infertility, and growth deficiency. The main purpose of this study was to determine the different types of chromosomal anomalies in 1730 ID and/or MCA cases, who were referred by clinicians for cytogenetic assessment, in the northwest of Iran. To date, no similar study has been performed in this region. Frequency of ID in the general population: In developed countries is thought to be in order of 23.0%,

a. 0.3-0.5% for moderate and severe ID intelligence quotient [(IQ) < 50, 1-3% for mild ID (IQ 50-70)], 25-50% of severe ID cases

b. Possible sources for causes of ID: prenatal chromosomal, maternal infections, environmental factors, unknown influences; perinatal (during birth) gestational disorders, neonatal complications; and postnatal (after birth) infections and intoxicants, environmental factors.

c. Genetic forms of ID subdivided into two major categories: Syndromic ID-Characterized by associated clinical, radiological, metabolic or biological features; and non-syndromic (or none-specific) ID - In which cognitive impairment represents the only manifestation of the disease.

Methods

From January 2010 to December 2013, a total of 1,730 patients were referred to the Ibn Sina Medical Genetic Tabriz Center, Iran-Outpatients Clinic of Tabriz University of Medical Sciences for chromosomal analysis on the basis of symptoms of ID and/or MCA by neurological specialists. There were 788 males and 942 females, and the average age of the patients was 13.5 years (age range: 0-40 years). The subjects had unknown reason of ID and/or MCA as well as some of them presented family history of ID, developmental retardation, skeletal abnormality, growth retardation, abortion and infertility/sub-infertility indicative of a familial chromosomal inversion/translocation. The IQ of the ID patients was below 70, thus characterizing the ID subjects based on Diagnostic and Statistical Manual of Mental Disorders-4th Edition (DSM-IV) criteria.

This study was approved by the Ethical Committee of Tabriz University of Medical Sciences and informed consent for genetic karyotyping was received.

Blood samples were collected and metaphase chromosome spreads were prepared from phytohemagglutinin (PHA)-stimulated cultures of peripheral blood lymphocytes at the 800-1000-band level using standard cytogenetic methods.[3-6]

Briefly, white blood cells (WBCs) were cultured in 10 ml Roswell Park Memorial Institute (RPMI) 1640 (Gibco®; Invitrogen) media, complemented with 20% (v/v) fetal bovine serum (FBS) (Gibco®; Invitrogen) and 10 µl/ml PHA (Gibco®; Invitrogen), at 37 °C for 72 hours. Thymidine (300 µg/ml) was added to the cultures after about 48 hours from the start of culture. After centrifuging for 16 hours at 1000 g, the supernatant was removed and the cells were washed once with 10 ml of fresh medium. Then, the

culture was restarted completely afresh for further 4.5 hours, after which 20 µl of colcemid was added 30 minutes before harvesting following the conventional blood cultures protocol as described.[2] Then, the suspension was centrifuged, and the pellet was resuspended in 5-10 ml of potassium chloride (0.075 M) and again centrifuged for about 20 minutes at 37 °C. Since centrifugation, the cells were resuspended in fixative (3 Vol methanol: 1 Vol acetic acid) (Merck, Frankfurt, Germany). The fixative was varied at any rate three times. By using a Pasteur pipette, a drop was dropped onto the slide level. The chromosomes were observed under phase contrast microscope to evaluate quality of the metaphases and the nuclei. The chromosomes were treated with trypsin and then stained with Giemsa stain after ageing. GTG high resolution–banded chromosomes were analyzed 20 random metaphases spreads from two independent cultures photographing four metaphase spreads for karyotyping (karyotyping system Ikaros, MetaSystem, USA) according to the International System for Human Cytogenetic Nomenclature criteria. In cases with karyotype mosaicism at least 50 random metaphases were assessed, and the parents of subjects with structural chromosomal abnormalities were karyotyped.

Results

Cytogenetic analysis of high-resolution metaphases from 1730 individuals showed that chromosomal anomalies occurred in a total of 440 (25.5%) of the 1730 patients (Table 1). The most common reason for requesting cytogenetic testing was mental retardation. The frequency of structural abnormalities with 70.0% of total abnormalities is much more than structural abnormality. The highest frequency of abnormal karyotype with 14.0% of total subjects is belong to chromosome 21. In almost all cases the origin of abnormal chromosomes are de nova. Marker chromosomes with an unknown origin were identified in eight de novo cases, and some patients showed other balanced and unbalanced chromosomal rearrangements (Table 1). 20 cases had an inversion chromosome 9, which is generally believed to be a normal variant, but eight female cases with this karyotype had two or three miscarriages. In 46 males, a positive fragile X anomaly was found (6.5% of all the screened male cases and 16.0% of the male cases with chromosome abnormalities).

Discussion

The etiology of developmental delay and ID or intellectual delay may be identified with a detailed history and physical examination. The evidence strongly suggests that in the absence of a clinical diagnosis after history and physical examination, routine screening of all these individuals should include chromosome karyotyping, FID1 gene testing and neuroimaging. In the genetic laboratory, karyotyping is routinely used for identifying unknown ID and/or MCA. Approximately, 1 in 200 babies is born with a chromosomal abnormality.[7]

However, only 30.0% of these cases are properly diagnosed. ID and/or MCA most probably is the consequence of the monogenetic and chromosomal disorders.[8] Identification of the critical region in the chromosomes is very useful in providing genotype-phenotype correlations and in defining a particular genetic syndrome. Most documented congenital malformations have been strongly associated with chromosomal anomalies, based on the patient's family history and clinical phenotype, cytogenetic workups are often recommended to arrive at a precise diagnosis. In general, a routine chromosome analysis must be used as an initial point for any cytogenetic analysis for developmental delay and/or ID.[9] Depending on the clinical symptoms, extra-chromosomal counts may be needed for rejection of mosaicism, and suitable band levels should be reached to detect small aberrations in the targeted areas.[10-12] Several cytogenetic studies on ID and/or MCA have been conducted in various regions around the world.[13-18]

Table 1. Cytogenetic results for 1730 patients

Chromosomal anomaly	Karyotype	Number of cases	Percentage	Sex
Down syndrome	47, XX + 21	172	13	64 F/108 M
	47, XY + 21			
	47, XX-14 + t(14q21q)	8	0.62	5 F/3 M
	47, XY-14 + t(14q21q)			
Edward syndrome	47, XY + 18	8	0.652	3 F/5 M
	47, XX + 18			
Patau syndrome	47, XY + 13	4	0.31	4 M
Turner syndrome	45, X	16	1.24	15 F/1 M
	45, X/46, XX	20	1.55	20 F
	45, X/46, X, isoX	4	0.31	4 F
	46, XX/46, X, isoX	8	0.62	8 F
Klinefelter syndrome	47, XXY	32	2.48	32 M
Super man	47, XYY	8	0.62	8 M
Super woman	47, XXX	4	0.31	4 F
Testicular feminization syndrome	46, XY	8	0.62	8 M
Balanced translocation	46, XY + Invchr. 1	4	0.31	4 M
	46, XX, Inv(.9)(p11q13)	7	1.55	16 F/4 M
	46, XY, Inv(.9)(p11q13)	9		
	46, XX, Inv(.9)((q31q34)	1		
	46, XY, Inv(.9)((q31q34)	3		
	46, XX/46, XX + t(7;14)	4	0.31	4 F
	46, XX + t(5;21)	4	0.31	4 F
	46, XY + t(15;16)	8	0.62	8 M
	46, XY + t(10;11)	12	0.93	12 M
	46, XX + t(7;14)	4	0.31	4 F
Marker chromosome	47, XY + Mar(15)	8	0.62	8 M
	47, XY + Mar(21)			
Other translocation and unbalanced chromosomal competitions	46, XX, del(5p)	4	0.31	4 F
	46, XY, dup(15p)	4	0.31	4 M
	46, XX, del(22p)	12	0.93	4 F/8 M
	46, XY, del(22p)			
	46, XY, dup(6q)	4	0.31	4 M
	46, XY, dup(16q)	4	0.31	4 M
	46, XY, ins(11q)	4	0.31	4 M
	46, XX/47,XXX	4	0.31	4 F
	46, X del(Xq)	8	0.62	8 F
	46, X del(Yq)	8	0.62	8 M
	46, X dup(Yq)	4	0.31	4 M
	46, X del & iso(Yq)	4	0.31	4 M
	46, XY sat(13)	4	0.31	4 M
	46, XY sat(14)	10	0.77	4 F/6 M
	46, XX sat(14)			
	46, XY sat(21)	6	0.56	6 M
	46, XY sat(22)	2	0. 15	2 M
Triploid	69, XXX	2	0. 15	2 F
Normal	46, XX	1290		611 F/679 M
	46, XY			
Total		1730		440

M: Male; F: Female

In this study, the frequency of Down syndrome (DS) was 41.0% in ID children, indicating a higher frequency and the need for prevention through prenatal diagnosis. The presence of trisomy 21 was observed in 96.0% of the patients, whereas Robertsonian

translocations were detected in 4.0% of the study population, supporting the reports of published literature.[19]

Missing or extra sex chromosomes (X and Y) affect sexual development and may cause infertility, development anomalies as well as social and educational problems. The sex chromosome-related syndromes commonly occurred with numerical anomalies such as Turner syndrome, Klinefelter syndrome, XXX syndrome and XYY syndrome, and this finding is in concordance with previously reported studies. The high-resolution banding method employed in this study was helpful in the diagnosis of various deletion syndromes and in establishing genotype-phenotype correlations. Translocations observed in 14.5% of the cases were either Robertsonian or reciprocal and were either balanced or unbalanced. The association of translocations with poor obstetric history, fertility failure, amenorrhea, ambiguous genitalia and ID with MCA or DS has been well documented by various researchers.[20,21]

Clinically, the most important single Robertsonian translocation involves chromosomes 14 and 21 (detected in two patients in this study); 4.0% of patients with DS are associated with this translocation.

The other Robertsonian translocation involves chromosome 14, which occurs less frequently than that involving chromosome 22. Sporadically, four female chromosomes 21 undergo a balanced translocation, 46,XX_+ t(5; 21) karyotype, has been observed in our study. Those women have 2 or three miscarriage. Robertsonian translocations between chromosomes 14 and 21 are of particular clinical interest, and these chromosomes are the most frequently involved in fusion (about 1 in 1000 newborns). An individual with this translocation could have a child with three copies of chromosome 21, resulting in DS (trisomy 21). Women who carry this translocation have a 10-15% risk of giving birth to a baby with DS, whereas men who carry this translocation have 1-3% of similar risk. No correlation between age and DS coupled with a translocation has been reported, but a parent with a balanced Robertsonian translocation that already has DS child has a relatively higher risk (10-30%) of having affected children. When balanced chromosome anomalies occur in parents (in the clinically silent form), they also have the potential of generating offspring with unbalanced karyotypes and birth defects. Therefore, when a translocation is detected after a pregnancy loss, parental karyotypes must be assessed to determine whether it is inherited or de novo in origin.

Other common translocations include t(5;21), t(7;14), t(10;11), and t(15;16), which have been extensively described in the literature, whereas some are associated with limited published reports.

Although translocations have been strongly associated with male infertility and recurrent spontaneous abortions, their relationship with ID remains elusive. Structural anomalies involving the Y chromosome do not lead to specific ID syndromes but are of great significance in male infertility. Salo et al. reported a sex chromosome constitution of 46, X, small Y in ID patients with dysmorphic features.[22]

Conversely, Hou and Wang[23] examined Y chromosome polymorphisms in Taiwan, citing no indications that Yq+, invY, and Yq− were related to any deviation in intelligence or with an increased risk for physical malformations or other chromosomal disorders. Current studies have later confirmed these results. In our study, four males with ID or fertility problems showed a small Y, long Y, isochromosome Y, which can only be further examined using the latest molecular techniques such as multiplex polymerase chain reaction analysis.

Structural and numerical aberrations involving both paracentric and pericentric inversions of chromosome 9 have been well established among subjects with ID. Most of the reported chromosome 9 inversions do not give rise to any specific phenotypic anomalies. However, a few cases have been associated with infertility, repeated fetal loss,

congenital anomalies and ID, possibly as a predisposing factor for nondisjunction and interchromosomal effect.[24,25] In this study, most of chromosome 9 inversions were pericentric and four subjects were paracentric. Only two cases phenotypically affected by infertility and no one compatible with ID.

Knowledge of the critical regions in chromosomes is very useful in establishing genotype-phenotype correlations. Karyotyping determines these critical regions and thus numerous congenital malformations could be linked to specific chromosomal aberrations. Genetic diagnosis by cytogenetic screening thus proves to play an essential role in the counseling of parents. The karyotype of parents with chromosomally abnormal children could help in the establishment of the inheritance pattern or the recurrence risk in a family and assist in its prevention and genetic counseling. However , since a routine cytogenetic analysis gives a minimum resolution of only 4-10 Mb, other advanced molecular cytogenetic techniques would be helpful for the diagnosis of the ID patients with normal karyotype as mentioned by some researcher.[12,26]

We compared our result to similar studies from northwest of Iran and southeast of Turkey.[27] We selected these two population because similarity of genetic base, religion, and geographical location. We had 25.5% abnormal cases in contrast to 16.7% in the northeast of Iran and 32.2% in Turkey. DS was the most prevalent autosomal aberration in the patients, 77.1% in northeast of Iran, 84.8% in Turkey and 41.0% in our study in northwest of Iran. Sexual chromosome abnormalities in northwest of Iran is 12.71% in contrast to the northeast of Iran with 24.9 and 17.6% in Turkey, that indicates lower rate. In all populations, Kline filter was the prevalent sexual chromosome abnormality.[27,28]

Conclusion

This study shows that chromosomal analysis is an important primary diagnostic tool for children with ID, dysmorphic features and developmental delay, which in turn will facilitate suitable genetic counseling, as well as the special education and management of ID and/or MCA children. These findings emphasize the usefulness of cytogenetic investigation in ID and/or MCA parents and the importance of increasing physicians' awareness regarding its importance.

Conflict of Interests

Authors have no conflict of interest.

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