

Chromosomal aberrations in mental retardation: A preliminary study

Debasis Bandopadhyay^{1,*}, Sushil Kumar²

¹Associate Professor, ²Professor & HOD, Dept. of Anatomy, Armed Forces Medical College, Pune, Maharashtra

*Corresponding Author:

Email: debasis13@hotmail.com

Abstract

Introduction: Chromosomal anomalies that change developmental gene expression are the most common cause of mental retardation. Roughly about 10% of all mental retardation cases suffer from chromosomal abnormalities. Sub-telomeric regions of chromosomes are gene-rich regions where rearrangements and deletions can cause mental retardation which accounts for nearly 2.5% of mental retardation cases, with or without dysmorphic features. Our study focuses on mental retardation likely to be due to chromosomal aneuploidies and large structural defects. These cases were detected using conventional cytogenetic technique by peripheral blood karyotype. A total of 30 cases of mental retardation as per exclusion/inclusion criteria were referred for genetic analysis to Cytogenetic lab, Dept of Anatomy, from May 2013 to Apr 2016. A peripheral blood karyotype was carried out in all patients.

Materials and Method: 5ml of venous blood was cultured for leucocytes and subsequently karyotyped using standard protocol of Trypsin Giemsa banding. The slides were visualised for metaphase spread under oil immersion and 20 cells were captured in every case for analysis using Cytovision software.

Results: Cytogenetic analysis of peripheral blood of 30 cases of mental retardation revealed 02 Down Syndrome pure cell line, 02 Down syndrome mosaic males, 01 Down syndrome female with 14/21 translocation, 01 Fragile X Syndrome male and 24 cases with normal karyotype.

Discussion & Conclusion: In our study only 06 out of 30 cases of mental retardation showed chromosomal anomaly analysed by peripheral blood karyotype. Previous data has concluded that genetically determined mental retardation aetiology is found in less than half the number of cases. Chromosomal aberrations, single-gene disorders, and other genetic conditions account for 17 to 41% of genetic cases of MR, even with newer techniques of genetic analysis.

Karyotype is gold standard investigation for aneuploidies and large structural defects however small deletions and alterations on chromosome are often missed in conventional cytogenetic procedures. Hence it is suggested that newer molecular cytogenetic techniques like Microarray and purely molecular techniques like Polymerase Chain Reaction (PCR) be used to diagnose submicroscopic aberrations which account for majority of aetiologically undiagnosed cases of mental retardation.

Keywords: Mental retardation, Mosaic, Karyotype, Microarray, Polymerase Chain Reaction

Introduction

Intelligence quotient (IQ) is a score attained from one of the various standardized tests designed to assess intelligence. Earlier IQ was described as ratio IQ calculated as person's mental age divided by chronological age multiplied by 100. Presently IQ is calculated as "deviation IQ" thereby defining mental retardation (MR) as two standard deviation below the mean of the general population somewhere below the 3rd percentile. The psychiatrists prefer using the term intellectual disability instead of mental retardation. Mental retardation has 3 important components of below average intellectual functioning (diminished ability to learn, reason, understand and solve problems), impaired adaptive behavior and onset before the age of 18yrs.

Chromosomal anomalies that change developmental gene expression are the most common cause of mental retardation.⁽¹⁾ Roughly about 10% of all mental retardation cases suffer from chromosomal abnormalities. The American Association of Mental Retardation classifies MR based on severity ranging from borderline MR (Deviation IQ 70-80) to profound MR (Deviation IQ below 25). Though mild and severe MR does not form two different aetiologically distinct

categories, available data shows that nearly 40% severe MR cases suffer from some form of chromosomal abnormalities whereas only about 10% mild MR cases show detectable chromosomal aberrations.⁽²⁾ Sub-telomeric regions of chromosomes are the most gene-rich regions and cryptic changes here in the form of rearrangements (unbalanced) and deletions account for nearly 2.5% of mental retardation cases, with or without dysmorphic features. An identifiable cause is established (genetic or environmental) in about 64% of the group with IQ less than 50, but this figure drops to approximately 24% in the IQ 50-70 group.⁽³⁾ Among known causes, the two largest individual contributors to mental retardation with chromosomal anomaly are Down syndrome and the fragile X syndrome. Over 500 other genetic diseases, mostly very rare, have also been associated with mental retardation and it is reasonable to suppose that a considerable proportion of cases of unknown aetiology have a genetic origin.^(3,4) In short genetic defects may account for over half of idiopathic mental retardation where the IQ is less than 50. Available database is not sufficient to estimate a similar figure for the IQ 50-70 group, but there are strong chances that single gene disorders and chromosomal

abnormalities may be more frequent than previously assumed.

Our study focussed on chromosomal aneuploidies and large structural defects in chromosomes in mental retardation detected using conventional cytogenetic technique by peripheral blood karyotype. A total of 30 cases of mental retardation were referred for genetic analysis to Cytogenetic lab, Dept of Anatomy, from May 2013 to Apr 2016. A peripheral blood karyotype was carried out in all patients. Exclusion criteria included all other causes of mental retardation (autism, cerebral palsy and other causes consequent to prenatal, perinatal and postnatal aetiologies like prematurity, low birth weight, preeclampsia, birth anoxia, infections, trauma and drug abuse) and single gene disorders/Mendelian inheritance.

Materials and Method

From every patient 5 ml of sterile venous blood was collected in heparin for cytogenetic analysis after taking informed consent from the patients. The venous blood was collected in a heparinized syringe from the cubital vein of the patients and was allowed to stand at room temp for 2-3 hours till a clear separation of the plasma with a buffy coat of lymphocytes was seen. Under aseptic conditions the plasma rich in lymphocytes was planted in the culture medium PB MAX GIBCO (Peripheral blood readymade culture media enriched with phytohaemagglutinin and L-Glutamine solution) and cultured for 70 h at 37°C in a culture vial / a centrifuge tube. The cells were then harvested by adding 0.1ml colcemid (10µgm/ml) to arrest the dividing cells at metaphase. After 2h the culture was centrifuged at 1000rpm for 10min, supernatant was discarded and 5ml hypotonic solution (0.56gms KCL in 100ml distil water) was added. This mixture was incubated at 37°C for 40 min followed by addition of 2-3 drops of freshly prepared fixative (Methanol and Acetic acid in 3:1 proportion) and centrifugation at 1000rpm for 10 mins. The supernatant was discarded and 5ml of freshly prepared chilled fixative was added to each tube and kept overnight at 4°C. Next morning 3 washes were given in fixative till the cell pellet was white in colour. These cells were then dropped on the slides and allowed to dry over moist heat at 75°C followed by overnight aging at room temperature. The slides were then treated with Trypsin (0.02gm in 90ml Phosphate Buffer Solution) and subsequently stained with Giemsa stain for G-Banding. The metaphase spreads were viewed under a microscope and at least 20 good spreads were captured for analysis using an image analyzer (Cytovision from Leica Biosystems).

Results

The karyotype analysis of the 30 cases is tabulated in Table 1.

Table 1: Karyotype findings in 30 cases of mental retardation. 24 cases were found to have normal karyotype and will further require molecular gene analysis to reach a genetic diagnosis

Sl No.	Clinical Diagnosis	Number of cases	Karyotype
1.	Down Syndrome pure cell line	02	47, XX+21
2.	Down Syndrome mosaic	02	1. 47, XY+21 (90%); 46, XY (10%) 2. 47, XY+21 (75%); 46, XY (25%)
3.	Down Syndrome translocation	01	46, XX, rob(14;21)(q10;q10),+21
4.	Fragile X Syndrome	01	46, Y, fra(X){q27.3}
5.	Mental retardation	24	1. 46, XY Normal karyotype – 10 cases 2. 46, XX Normal karyotype – 14 cases

The Fig. 1 shows the karyotype of Down Syndrome female with pure cell line.

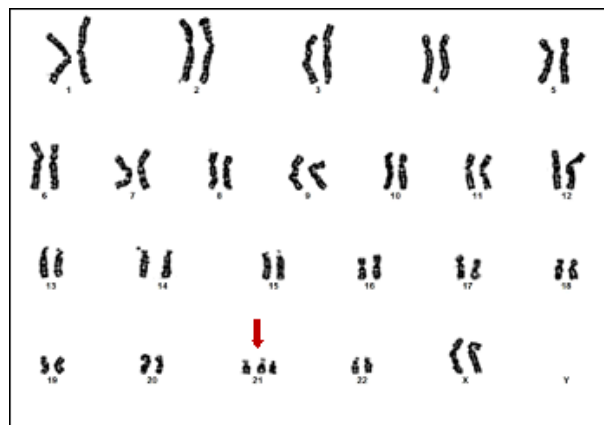
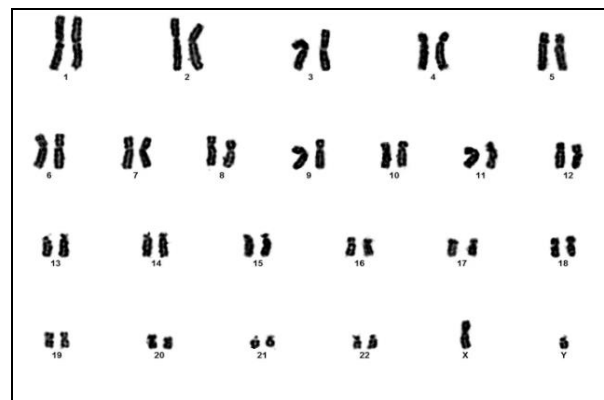


Fig. 1: 47, XX+21 karyotype seen in Down Syndrome with pure cell line

The Fig. 2 shows the karyotype of Down Syndrome male with mosaic cell line.



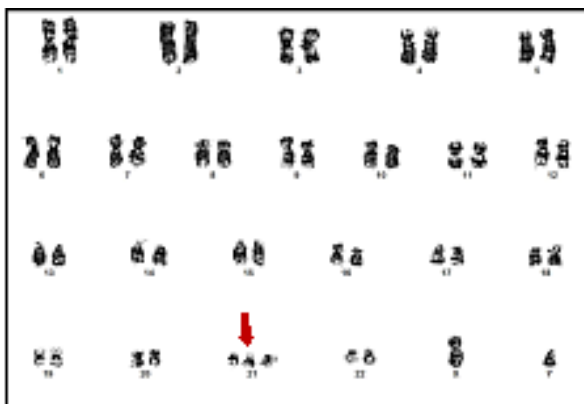


Fig. 2: 47,XY,+21 (90%); 46, XY (10%) karyotype seen in Down Syndrome mosaic male

The Fig. 3 shows the karyotype of Down Syndrome female with 14, 21 Robertsonian translocation.

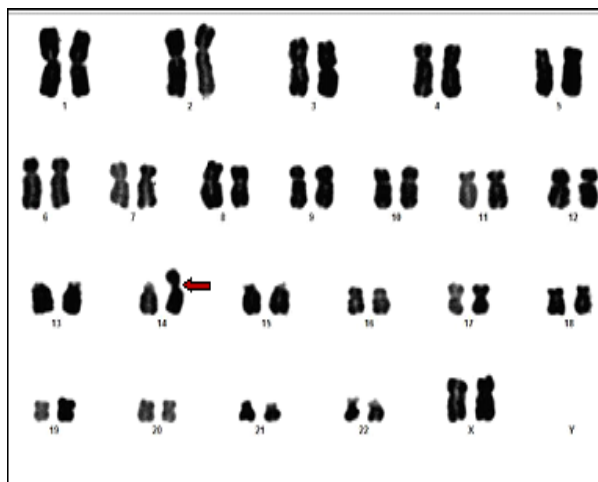


Fig 3: 46,XX,rob(14;21)(q10;q10),+21 karyotype seen in Down Syndrome female

The Fig. 4 shows the karyotype of Fragile X Syndrome in male.

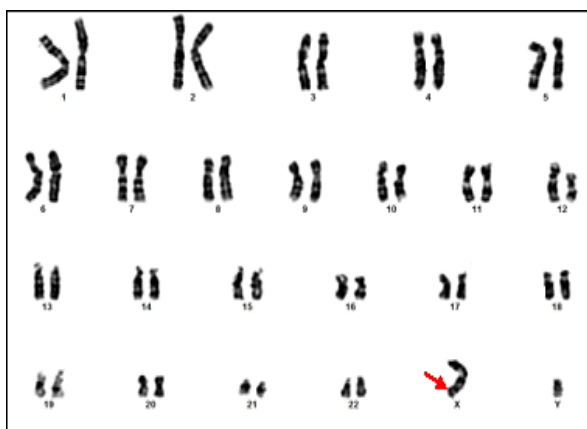


Fig. 4: 46,Y,fra[X]{q27.3} karyotype seen in Fragile X Syndrome (male)

Discussion

Mental retardation or intellectual disability has a prevalence of 2-3% in the general population and is a heterogeneous neuro-developmental disorder numerically defined by an intelligence quotient (IQ) of less than 70.⁽⁵⁾ A genetic aetiology for mental retardation has been long recognized ever since the discovery of Down syndrome as trisomy 21 in 1959.⁽⁶⁾ Thereafter the research has been oriented to identify smaller and smaller chromosome abnormalities associated with MR. Numerical and structural abnormalities are responsible for about 4-28% of all mental retardation with incidence of 40% in severe mental retardation and 10% in mild mental retardation.⁽²⁾ Numerical anomalies affect autosomes more often than sex chromosomes, with a median frequency of 6.5% vs. 0.4%. Numerical anomalies of the sex chromosomes occur foremost in borderline to mild mental retardation, while numerical anomalies of the autosomes are mostly detected in patients with more severe mental retardation. Structural chromosome anomalies usually not smaller than 5 Mb, are detectable using cytogenetic studies with 400- 450 bands resolution in giemsa trypsin banding.^(7,8)

We analysed 30 cases of mental retardation with / without dysmorphic features by peripheral blood karyotype to look for numerical/ structural aberrations of chromosomes. There was no severity based grading of mental retardation. All cases of autism, cerebral palsy and single gene disorders causing mental retardation were excluded. The cytogenetic analysis revealed 02 cases of Down Syndrome pure cell line, 02 cases of Down Syndrome mosaic, 01 case of unbalanced Robertsonian translocation 14, 21 in Down Syndrome, 01 case of Fragile X syndrome and 24 cases of normal karyotype. The incidence of chromosome abnormality reported by various authors includes mental retardation patients with both consanguineous and non-consanguineous parents. In our study all couples were non-consanguineous parents and there was no family history of mental retardation.

Many of the patients referred to us for Fragile X syndrome had typical facial appearance but was found to have normal karyotype. In most of these patients FMRI gene analysis for CGG triplet repeats gave positive results. A 55-200 CGG repeats is suggestive of permutation and above 200 CGG repeats suggests full mutation. Hence in Fragile X syndrome karyotype which was gold standard investigation prior to 1990 has been replaced by molecular gene analysis methods detecting 99.99% cases of FMR1 gene mutation.⁽⁹⁾

The MR patients referred to us with dysmorphic features suggestive of Down Syndrome were found to have trisomy 21 in 100% of the cases. In 2 cases of mild MR and no dysmorphic features suggestive of Down Syndrome karyotype revealed mosaic cell line with varying percentage of trisomy 21 and normal cell line. Hence even today Karyotype remains the Gold

standard investigation for Down Syndrome. Reliable molecular methods like Fluorescent in Situ Hybridization (FISH) and Quantitative Fluorescence Polymerase Chain Reaction (QF-PCR) are available for rapid diagnosis but are still not recommended as standalone investigation for Down Syndrome and are required to be followed up by karyotype.⁽¹⁰⁾

Sub-telomeric rearrangements account for 2.5% of idiopathic MR but difficult to detect on 450-550 band karyotype resolution. The cryptic changes in these regions can be best picked up by high resolution banding and molecular techniques like PCR and gene sequencing.⁽¹¹⁾

Genetically determined mental retardation aetiology is found in less than half the number of cases. Chromosomal aberrations, single-gene disorders, and other genetic conditions account for 17 to 41% of genetically attributable cases of MR, even with newer techniques of genetic analysis. Karyotype remains gold standard investigation for aneuploidies and large structural defects however small deletions and alterations on chromosome are often missed in conventional cytogenetic procedures. With the advent of novel genetic techniques, several new cryptic chromosomal aberrations have been discovered in last few years and a consistent number of MR cases, previously considered "idiopathic" forms, are now classified as syndromic conditions with clinical recognizable phenotypes.^(12,13) Hence it is suggested that newer molecular cytogenetic techniques like Microarray and purely molecular techniques like Polymerase Chain Reaction (PCR) be used to diagnose submicroscopic aberrations which account for good chunk of aetiologically undiagnosed cases of mental retardation. The more detailed analysis employing special techniques like subtelomere fluorescent in situ hybridization, comparative genomic hybridization, or spectral karyotyping may help in determining the actual frequency of chromosomal abnormalities in the mental retardation cases.

Conclusion

Genetic abnormalities are the most common identifiable cause of unexplained mental retardation but conventional karyotyping is unable to detect imbalances smaller than about 3-5 Mb. Though karyotype remains the mainstream investigation for aneuploidies and large structural defects in MR, the smaller chromosomal abnormalities is better identified with fluorescent in situ hybridization (FISH) or multiplex ligation-dependent probe amplification (MLPA) techniques, rapidly confirming a clinical diagnosis of well-known microdeletion/ microduplication syndrome. PCR and gene sequencing on other hand will provide information on any cryptic changes in subtelomeric regions of all chromosomes. The combined analysis of karyotype and subtelomeric regions, using FISH or other molecular techniques, have improved detection of chromosome

abnormalities by 5-10% in these patients previously diagnosed as idiopathic MR.

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