Cytogenetics study in severely mentally retarded patients

Akeel A. Yasseen, MSc (UK), PhD (UK), Thekra A. Al-Musawi, MSc.

ABSTRACT

Objectives: To study the frequency of chromosomal abnormalities in severely mentally retarded Iraqi patients. Secondly, to determine the types of chromosomal abnormalities that play a major role in the causation of mental retardation and to compare our results with those reported elsewhere.

Methods: Twenty one patients with severe mental retardation were subject to chromosomal analysis. The lymphocyte culture was carried out according to standard methods.

Results: Fourteen of the subjected mentally retarded patients had chromosomal abnormalities, 13 autosomal abnormalities, and only one had sex chromosomal abnormalities. However, structural autosomes were found to be the most prominent abnormalities and only 2 patients were demonstrated to have diagnosable syndrome.

Conclusion: Chromosomal abnormalities are an important cause of mental retardation and its frequency increased with the severity of mental retardation. We concluded that chromosomal studies in mentally retarded patients help in accurate diagnosis and proper prognosis followed by genetic counseling and management rehabilitation.

Keywords: Diabetes mellitus, glycemic control, primary health care.

Saudi Med J 2001; Vol. 22 (5): 444-449

Mental retardation (MR) is a common and distressing disorder whose origins are poorly understood. However, it is well documented that about 3% of the population has an intelligence quotient (IQ) less than 70,[1] yet a cause for MR is established in less than half of all cases.[2,3] The fact that a cause and an affect relationship between abnormal phenotypes and karyotypes were documented by the Madison blind study which showed that the unbalanced euchromatic abnormalities were present in phenotypically abnormal individuals and absent in normal ones.[4] Since then various surveys were carried out on karyotype patients with MR that clearly revealed MR patients with associated congenital abnormalities had a higher frequency of chromosomal abnormalities than those without.[5] Interestingly, it is well demonstrated that chromosomal disorders are often the cause of MR of different severity, but the origin and nature of this relation is far from clear.[6] Indeed, chromosomal abnormalities are thought to have their effect by altering gene dosage by which, in the simplest scenario, autosomal deletion halve gene expression in the monosomic region while in trisomic there is over expression by 50% or more.[7] So the larger the chromosomal abnormalities, the more severe phenotype, but sometimes the picture is more complicated. However, both types of chromosomal abnormalities, the numerical and the structural that affect the autosome, as well as the sex chromosomes

From the Department of Pathology and Forensic Medicine, College of Medicine, Kufa University, Kufa, Iraq.

Received 26th August 2000. Accepted for publication in final form 22nd January 2001.

Address correspondence and reprint request to: Dr. A. Yasseen, Medical Genetics, Department of Pathology and Forensic Medicine, College of Medicine, Kufa University, PO Box 18, Kufa, Iraq. Fax +964 (1) 886 7170.

Table 1 - Detailed description of 21 mentally retarded patients.

No. of patients	Age (Year)	Sex	Consanguinity	Maternal	Paternal	Brief family history
1	9	F	First-degree	36	39	It is negative for any birth defects or mental retardation
2	17	M	Non	35	40	Negative
3	17	M	Second- degree	45	48	His sister was mentally retarded
4	14	M	Non	38	35	Negative
5	8	F	Non	32	39	Her sister was retarded patient
6	19	M	Second-degree	16	25	Negative
7	18	M	First-degree	17	26	His grandfather and other family members were mentally retarded
8	12	M	Non	44	45	Negative
9	10	M	Non	35	35	Negative
10	10	M	First-degree	28	29	Negative
11	16	M	Second-degree	25	32	Negative
12	8	F	First-degree	34	35	Negative
13	16	M	First-degree	26	32	His uncle was retarded patient
14	18	M	First-degree	38	30	Negative
15	15	M	Non	35	40	Negative
16	18	M	First-degree	36	39	Negative
17	19	M	Non	40	41	Negative
18	4	F	Non	37	49	Negative
19	5	M	First-degree	28	39	Negative
20	5	M	Second-degree	33	35	His brother was mentally retarded
21	19	M	First-degree	36	46	Negative
Mean				33.04	37.09	
f-female, m-male, Sex ratio (4.3/1)						

have been recorded.[5,8] It is generally agreed that autosomal aberration are the single most common cause of severe MR[9] and also found that abnormalities in the number of sex chromosome, generally have less devastating effects than aneuploidies in the autosomes.[8] In other words, it has held that aneuploidy is the most frequent cause of MR[10] in which autosomal trisomies are more frequent than monosomies.[5] In view of this, the present study was designed to investigate the frequency and types of chromosomal abnormalities in 21 mentally retarded patients in Najaf province. To our knowledge, karyotype analysis on mental retardation has never been conducted in Iraq before the present report.

Methods. Twenty-one patients have been diagnosed and classified as severely mentally retarded patients, with a median age of (13.2) years (range 4-19) were subjected to the present study. The patients were randomly selected from Al-Rajaa institution for MR in Najaf province. All the patients who were clinically diagnosed as Down's syndrome were excluded. A detailed history and extensive pedigree were noted for each patient on a structured preplanned proforma (Table 1). Peripheral blood lymphocytes were cultured according to the standard method of Moorhead et al.[11] About 0.5 ml of heparinized venous blood was extruded in the culture tube, containing RPMI-1640 culture media supplemented with L-glutamine, 20% fetal calf

Table 2 - Types and frequencies of chromosomal abnormalities in mentally retarded patients from various surveys.*

Reference	Total patients examined	Sex chromosome abnormalities No (%)	Autosomal abnormalities No (%)	Total No (%)
Madison Blind study	240	2 (1)	20 (8)	22 (9)
Chen et al	134	6 (4)	6 (4)	12 (9)
Corey et al	223	5 (2)	10 (4)	15 (7)
Correl et al	121	1 (1)	18 (15)	19 (16)
Lubs and Lubs	54	0 (0)	4 (7)	4 (7)
Erdtmann et al	51	1 (2)	4 (8)	5 (10)
Coco and penchaszaden	131	2 (1.5)	26 (20)	28 (21)
Moghe study	74	7 (9)	7 (9)	14 (19)
Present study	21	1 (5)	13 (62)	14 (67)

*Moghe et al(1981)[5]

serum, 2% phatoheamagglutinin, 50 I.U/ml penicillin and 100 I.U/ml streptomycin. At the end of the incubation period (37 for 72 hours) the colchicine was added for a further 2 hours at a final concentration 0.004% w/v. Then, the cells were exposed to hypotonic shock 0.075 M kcl for 30 minutes and fixation in methanol: glacial acetic acid (3:1). The suspended cells were dropped from about a 30-40 cm height onto cold damp slides. The slides were dried in a stream of cold air and then stained with 10% (v/v) Giemsa in phosphate buffer saline for 10 minutes followed by a brief washing in running tap water. Finally, the slides were mounted in Depex after air-drying. In each case, a minimum of 30 metaphases was analyzed. Whenever mosaicism was detected a total of 50 cells were investigated to determine the degree of mosaicism. For each case, 10 well spreaded metaphases were photographed and karyotyped.

Results. Out of 21 severely mentally retarded patients that were recruited, 14 patients revealed obvious chromosomal abnormalities, which accounted for 67%. Of the established chromosomal abnormalities, 13 patients were found to have autosomal abnormalities, giving rise to a frequency of about 61% while 1 patient had sex chromosomal abnormalities giving rise to the frequency of about 5% (Table 2). Structural autosomes were detected in 12 patients which estimated to be 92% of the autosomal abnormalities and the rest (8%) were numerical autosomes (Table 3). The types of chromosomal abnormalities were summarized in Table 4. Karyotype investigation revealed that structural autosomes represent in ring, translocation (Robertsonian and non-Robertsonian), centromeric gap and secondary constriction was diagnosed in our 12 patients, while the numerical autosome was detected in only one case as trisomy 21 mosaicism. Sex chromosome abnormality was gonosome/ autosome translocation. Among previous patients, 2 demonstrated having multiple chromosomal abnormalities, involving translocation associated with centromeric gap in one case and translocation with chromatid deletion in another case. Two patients here were diagnosed as detectable syndrome (Down's syndrome) which associated with suldon chromosome constriction of 47,XY, +21/46,XY in one of them and 46,XX, +t(Dq;21q) karyotype in another.

Discussion. Several surveys have reported the frequency and types of chromosomal abnormalities in patients with MR. The frequency of chromosomal

Table 3 - Types of chromosomal abnormalities.

Abnormality	Structural No (%)	Numerical No (%)	Total No (%)
Autosomal abnormalities	12 (92)	1 (8)	13 (93)
Sex chromosomal abnormalities	1 (100)	0 (0)	1 (7)
Total	**13 (93)**	**1 (7)**	**14 (100)**

Table 4 - Types of chromosomal abnormalities for 20 severely mentally retarded patients.*

Patients	No of lymphocytes analyzed	Karyotype	Associated syndrome
Structural autosomes			
KA	50	46, XY, r (G)/46, XY, -	Non
AM	50	r(G)/46, XY	Non
RH	50	46, XX, r(D)/46,XX-(D)/46,XX	Non
HD	50	46, XY, r (D)/46, XY,-	Non
YA	50	r(D)/46, XY	Non
AH	40	46, XY, r(E)/46, XY,-(E)/46,XY	Non
DR	35	46, XY,r(E)/46,XY,-	Down's
AA	50	r(E)/46,XY	Non
HH	50	46, XY, t(Bq,Cq)	Non
AH	40	46, XX, t(Dq, 21q)	Non
		46, XY, Cqh+/46,XY	
Multiple chromosomal abnormalities	35	46, XY, ceng (C), 46, XY	Non
MA	40	46, XY, ceng (C)	Non
AZ			
Numerical autosomes	50	46, XY, ct de (Bq), t(Cq, Cq)	Down's
AY		46, XY, ceng (C), t (Cq, Eq)	
Sex chromosomal abnormalities	25	47, XY, +21/46, XY	Non
MH		46, XY, t (Cq, Yq)	

*The nomenclature used is according to the International system for Human Cytogenetic Nomenclature (1978).[12]

abnormalities in these surveys varied from 7% to 21%. In the present study the frequency of chromosomal abnormalities accounted for around 67%. The differences in frequencies might be due to countries where other studies were conducted on almost every mentally retarded patient, even those who showed few symptoms, were thought to be sent to special care institutions. Of course and as might be expected, the less mentally retarded patients, the less chromosomal aberration is expected to be found. In our cases, only the severe mentally retarded patients were sent to Al-Rajaa institution. So we expected to find much more of a frequency of chromosomal aberration than that published elsewhere due to the chromosomal anomalies being the single most common cause of severe MR.[9] However other investigators reported around 50% which is not really different from our study and consolidates our findings that in the severe mentally retarded the frequency of chromosomal abnormality is much higher than those who are less affected.[13] As presented in Table 2, the present investigation showed that sex chromosomal abnormalities are less frequent than autosomal abnormalities that account for 5% while the autosomal comprise of 62% of all 21 mentally retarded patients. These percentages seem to be in full agreement with those reported by other investigators. It is generally agreed that sex chromosome abnormalities are associated with slight decrease in the IQ and have generally less devastating effects than that of autosomal abnormalities.[8,14] However, the majority of our cases revealed structural autosomal abnormalities. Two patients with Down's syndrome who were associated with rare chromosome constitution have been detected. One patient showed 47,XY, +21/46,XY mosaicism (50:50) while the other showed 46,XX,t (Dq;21q) female mitotic karyotype. Obviously, the majority of Down's syndrome patients can be diagnosed with certainty from clinically signs and dermatoglyphics. Therefore, karyotyping is most often carried out to detect translocation cases for the

purpose of genetic counseling only. The possible explanation for the occurrence of trisomy 21 mosaicism is that the zygote started as normal 46, XY, non-disjunction at the second mitotic division resulted in a 46/47 mosaicism (non-disjunction at the first mitotic division would result in a regular trisomy), or the zygote started out as trisomic for a chromosome 21. Anaphase lagging or a chromosome 21 at one of the first mitotic divisions can also result in a 46/47 mosaic.[15,16] In another case of Down's syndrome, the translocated chromosome may be inherited from one of the carrier parent, or regarded as mutant Robertsonian translocation case. This explanation has been suggested by other reports which show that about 59% of D/21 cases are the results of mutation in the parental generation and the rest are translocation inherited from parental carriers (39% maternal, 3% paternal).[17]

Insight to our premention results which revealed interesting observations from the present study. Five patients (24%) showed a translocation rearrangement, Robertsonian and non-Robertsonian translocation and, further, they also showed a markered increase (19%) in non-Robertsonian translocation as has been shown elsewhere.[18] It seems likely that the combined incidence of non-Robertsonian balanced translocation from reported surveys of mentally retarded was 5 times more than that from newborn surveys, whereas Robertsonian were not increased among the retarded.[19] Our results support the view that autosomal translocation was found to be more frequent than the gonosome or autosome/gonosome translocation. Four cases with autosomal translocation and one case with autosome/gonosome translocation. The possible origin of this chromosome aberration were either de novo; familial rearrangement or of unknown origin. Usually, there is increase in de novo rather than familial rearrangement among the retarded that appeared to be specifically for non-Robertsonian translocation.[19] In addition, theoretically the Y chromosome has only genes which are responsible for male sex determination and growth promoting factors which are located on the large arm as well as the short arm, and as we expected the mental retardation in our autosome/gonosome translocation case result from disruption of gene(s) on the autosome due to translocation. On the other hand, ring chromosome appears to be the common abnormality among retarded patients, which was diagnosed in 5 cases. The ring formation results in a material loss of both the short and long arm ends. In general, the phenotypic effect might be attributed to the lost materials, as well as to the behavior of the ring chromosome in mitosis.[20] The lack of the acrocentric short arm material seems to be compatible with normal development,[21] therefore; the phenotypic effect in patients with acrocentric ring chromosome could be due to the variable deficiencies of the long arm and the composition of the mosaic with respect to the frequencies of the various ring configurations in the cells.[22] However, the ring chromosome are generally not associated with a constant and well-delineated phenotype that may be explained in part by differences in breakpoints followed by variable loss of material, and by mitotic loss of the ring chromosome in a variable percentage of cells at variable moments during embryogenesis.[23] In all our cases, mosaic form was observed in which chromosome constitution with ring chromosome was found to be more frequent while that of chromosome constitution without ring chromosome and that of normal karyotype were less frequent. This may be due to the fact that the chromosomal anomaly presumably was the result of post-zygotic error, the presence of the normal chromosome constitution shows that the ring originated after fertilization.[24] It is also clear that the presence of chromosome constitution without ring chromosome that is caused by the structural instability of the ring chromosome that is due to their specific behavior in mitotic anaphase.[24] Similarly, the proportion of cells whose rings were lost as a result of difficulties in anaphase variable greatly from case to case.[22] The fact that the heterochromatin is very likely to be affected in chromosomal abnormalities,[25] so the induced-damage occurred in centromere regions and secondary constriction more than other region observed in patients AA, HH, AH and AZ in our study. Such abnormalities might be arising from the effect of environmental mutagens that act during the early mitotic division after fertilization in the cases of mosaic form, or act during the first division of zygote or in the gametes of one parent in case of pure form.[26] Finally, the present study showed that two patients were found to have multiple chromosomal abnormalities. One of them has karyotype of translocation associated with centrometric gap and another case has a karyotype of translocation and chromatid deletion. Translocation and centrometric gap were discussed in some detail previously and, also we expected that the chromatid deletion caused by environmental mutagens during the mitotic division of zygote after fertilization.[26-27] However, multiple chromosomal abnormalities have been reported by many authors, although it is uncommon among the general population. In fact we can not deduce to certain the phenotypic effect and MR of our patients, whether they are as a result of the effect of one of these chromosomal abnormalities or that of accumulative effect of them.

Acknowledgment. The authors express sincere thanks to Al-rajaa institution in Al-Najaf province for their help in providing samples and data information about the patients.

References

1. Flint J, Willkie A, Buckle V, Winter R, Holland A, McDermid H. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. Nature Genet 1995; 9:132-140.
2. Gostason R, Wahlstrom J, Johannisson T and Holmqvist D. Chromosomal aberrations in the mildly mentally retarded. J Men Defic Res 1991; 35: 246-246.
3. Bienvenu T, Der-Sarkissian H, Billuart P, Tissot M, Portes V, Brüls T et al. Mapping of the X-Breakpoint in a balanced X; 2 translocation in a female with mild mental retardation. Eur J Hum Genet 1997; 5: 105-109.
4. Daly RF. Chromosome aberration 50 patients with idiopathic mental retardation and in 50 control subjects. J Pediatr 1970; 77: 444-453.
5. Moghe M, Patel ZM, Peter JJ, Ambani LM. Cytogenetic studied in a selected group of mentally retarded children. Hum Genet 1981; 58:184-187.
6. Gullotta F, Rehder H, Gropp A. Descriptive Neuropathology of chromosomal disorders in man. Hum Genet 1981; 57: 337-344.
7. Flint J, Wilkie A. The genetics of mental retardation. Brit Med Bull 1996; 52: 453-464.
8. Simonoff E, Bolton P, Rutter M. Mental retardation: Genetic findings, clinical implications and research agenda. J Child Psychol Psychiat 1996; 37: 259-280.
9. Gustarson KH, Holmgren R, Consell R, Son Blomquist HK. Severe mental retardation in children in a northern Swedish country. J of Men Defic Res 1977; 21: 161-180.
10. Griffin DK. The incidence, origin, and etiology of aneuploidy. Int Rev Cytol 1996; 167: 263-296.
11. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 1960; 20: 613-616.
12. An International System for Human Cytogenetic Nomenclature. Cytogen and Cell Genet 1978; 21: 313-404.
13. Hagberg B, Kyllerman M. Epidemilogy of mental retardation, a Swedish survey. Brain Devel 1983; 5: 441-449
14. De la Chapelle A. Sex chromosome abnormalities. In: Emery AEH, Rimoin DL (eds). Principles and practice of medical genetics. Edinburgh: Churchill-Livingston; 1990. p. 273-200.
15. Lieber E, Ince P. Tow Robertsonian translocations in a boy with mental retardation. J Med Genet 1982; 19: 229-232.
16. Soler A, Margarit E, Carrio A, Costa D, Queralt R, Ballesta F. Trisomy/tetrasomy 21 mosaicism in CVS: interpretation of cytogenetic discrepancies between placental and fetal chromosome complements. J Med Genet 1999; 36: 333-334.
17. Hooke EB. Unbalanced Robertsonian translocation associated with Down's syndrome or patau's syndrome: chromosome subtype, proportion inherited, mutation, rate and sex ratio. Hum Genet 1981; 59: 235-239.
18. Breg WR, Miller DA, Allerice PW, Miller OJ. Identification of translocation chromosomes by quinacrine fluorescence. Am J Dis Child 1972; 123: 561-564.
19. Funderburk SJ, Aune Spence M, Sparkes RS. Mental retardation associated with "balanced" chromosome rearrangements. AJ Hum Genet 1977; 29: 136-141.
20. Teyssier M, Piperno D, Charrin, C. Ring chromosome 3 in mentally retarded adult dwarf. Ann Genet 1991; 34: 33-36.
21. Nielson J, Friedrich V, Hreidarsson AB. Frequency of deletion of short arm satellites in acrocentric chromosomes. J Med Genet 1974; 11: 177-180.
22. Schimd M, Henrichs I, Nestler H, Knorr-Gärtner H, Teller W M, Krone W. Analysis of banding patterns and mosaic configuration in a case of ring chromosome 15. Hum Genet 1978; 41:289-299.
23. Fryns JP, Van den Berghe H. Ring chromosome 22 in a mentally retarded child and mosaic 45,XX,-15,-22,+t(15;22) (p11;q11)/46,XX,r(22)/46,XX karyotype in the mother. Hum Genet 1979; 47: 213-216.
24. Aller V, Abrisqueta JA, de Torres ML, Martin-Lucas MA, Perez-Castillo A, Delmazo J. An r(22) (p11Æq13) in a moderately mentally retarded girl. Hum Genet 1979; 51: 157-162.
25. Mattei MG, Mattei JF, Guichaoua M, Giraud F. Partial inversion of the secondary constriction of chromosome 9. Does it exist? Hum Genet 1981; 59: 310-316.
26. Fabricant J, Lagetor M. Etiology, role and detection of chromosomal aberration in man. J of Occup Med 1981: 23: 617-625.
27. Cano J, Caparros M, Pretel A. Comparative analysis of the rates of chromosome damage induced by bleomycine radiomimetic in human trisomic and diploid lymphocytes: in vitro cultures from a mosaic of a Down's syndrome individual. Cytobi 1995: 83: 159-166.