Clinical implication of Chromosomal Fragile sites in Down syndrome

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Abstract

Fragile sites /Chromosomal aberrations play a role in the etiology of mental retardation and other associated complications. Since Down syndrome is the most common genetic cause of mental retardation, the present study was done to evaluate the effect of fragile sites on sys-temic diseases and growth and developmental pattern among them. Human lymphocyte cell culture with folate free medium showed fragile sites in 22 cases among 40 investigated. Sys-temic examination and developmental delay were assessed according to standard protocol and compared with fragile sites . Fragile sites were associated with more severe mental re-tardation , reduced growth and increased systemic diseases among children with Down syndrome.

Key Words: Down syndrome (DS), Chromosomal Fragile Sites (CFS), developmental delay
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Introduction

Down syndrome (DS) is one of the most common genetic cause of mental retardation. It is due to extra genetic ma-terial that causes physical and mental retardation. The physical features and medical problems associated with Down syndrome vary widely from child to child due to the difference in gene expression. DS children are associ-ated with various complications like leukemia, Alz-heimer's and are more prone for infections [1]. Fragile sites /Chromosomal aberrations play a role in the etiology of mental retardation and other associated complications. Expression of these fragile sites depends upon various culture conditions [2]. Further, chromosomal fragile sites have been useful for mapping chromosomal regions of the genome that contain genetic loci important for the causa-tion of diseases and ageing [3].

Material and Methods

Forty children with clinical profile of Trisomy 21 and confirmed karyotypically in the age group ranging betw-een neonates to eleven years were investigated. Thorough clinical examination and developmental assessment were done. Denver developmental screening test was used for assessment. Anthropometric measurements were given a scoring based on the expected growth for a normal child of the same age and sex, since the cases were with vary-ing age group. Accordingly cases with 80% of normal growth were given score 10,between 70-80 % score ~~8, between 60-70 % score~~ 6, and below 60% score- 4.Conventional lymphocyte culture was carried out using RPMI 1640 and other media like folate free RPMI 1640 with 5-Azacytidine, a DNA methyl transferase inhibitor. Metaphase spreads were observed under Olympus BX51, Japan ,bright/ epiflorescence microscope and freezed with automated karyotyping workstation – Ikaros Metasys-tems, Carl Zeiss, Germany. AGT recommendations were followed for identification and interpretation of structural aberrations. The results were analysed using standard sta-tistical tests.

Results

Lymphocyte cell culture done using RPMI 1640 without folic acid and with 5-Azacytidine showed folate sensitive fragile sites in twenty two cases; of which thirteen were males and nine were females.

Systemic diseases were observed more often among cases with fragile sites than without disease (Table 1). Based on the scoring system used for analysing growth parameters, there was a significant decrease in the mean score with respect to height of cases with fragile sites when compared to those without fragile sites ($p < 0.05$). Though the mean score for weight and head circumfer-ence were not statistically significant, they were less in cases with fragile sites when compared to those without-fragile sites (Table 2).

Table 1: Association of fragile sites with systemic involvement

System involved	Cases affected	Cases with fragile sites	%	Cases not affected	Fragile sites	%
CVS	19	13	68.4	21	10	47.6
RS	16	12	75	24	10	41.6
GI	02	02	100	38	20	52.6
GU	02	02	100	38	20	52.6
Total	39	29	74.4	121	60	49.6

p value < 0.05

Table 2: Fragile sites with Growth parameters

Growth parameters	Mean score of cases with Fragile sites	Mean score of cases with-out fragile sites	P value
Height	6.81 ± 0.65	7.55 ± 0.61	<0.05
Weight	6.95 ± 0.65	7.38 ± 0.60	>0.05
Head circumf.	7.31 ± 0.77	7.50 ± 0.61	>0.05
Total Mean for growth parameters	7.02 ± 0.25	7.47 ± 0.08	>0.05

Mental retardation was categorised into mild, moderate and severe by taking mental age and chronological age into account (Developmental quotient). Fragile sites were significantly increased in cases with severe mental retar-dation as compared to that of mild and moderate .(Table 3)

Table 3: Developmental quotient correlated with presence of fragile sites

Degree of IQ	Total no of cases	No of cases with fragile sites	%
Mild (50-69)	19	05	20

Moderate (35-49)	09	06	55
Severe (<34)	12	11	91

Fragile sites were seen in A,B,C,D, groups of Denvers classification of chromosome. Fragile sites were seen in the form of breaks/gaps, triradial,quadriradial configura-tion,dicentrics and ring chromosome(Fig).

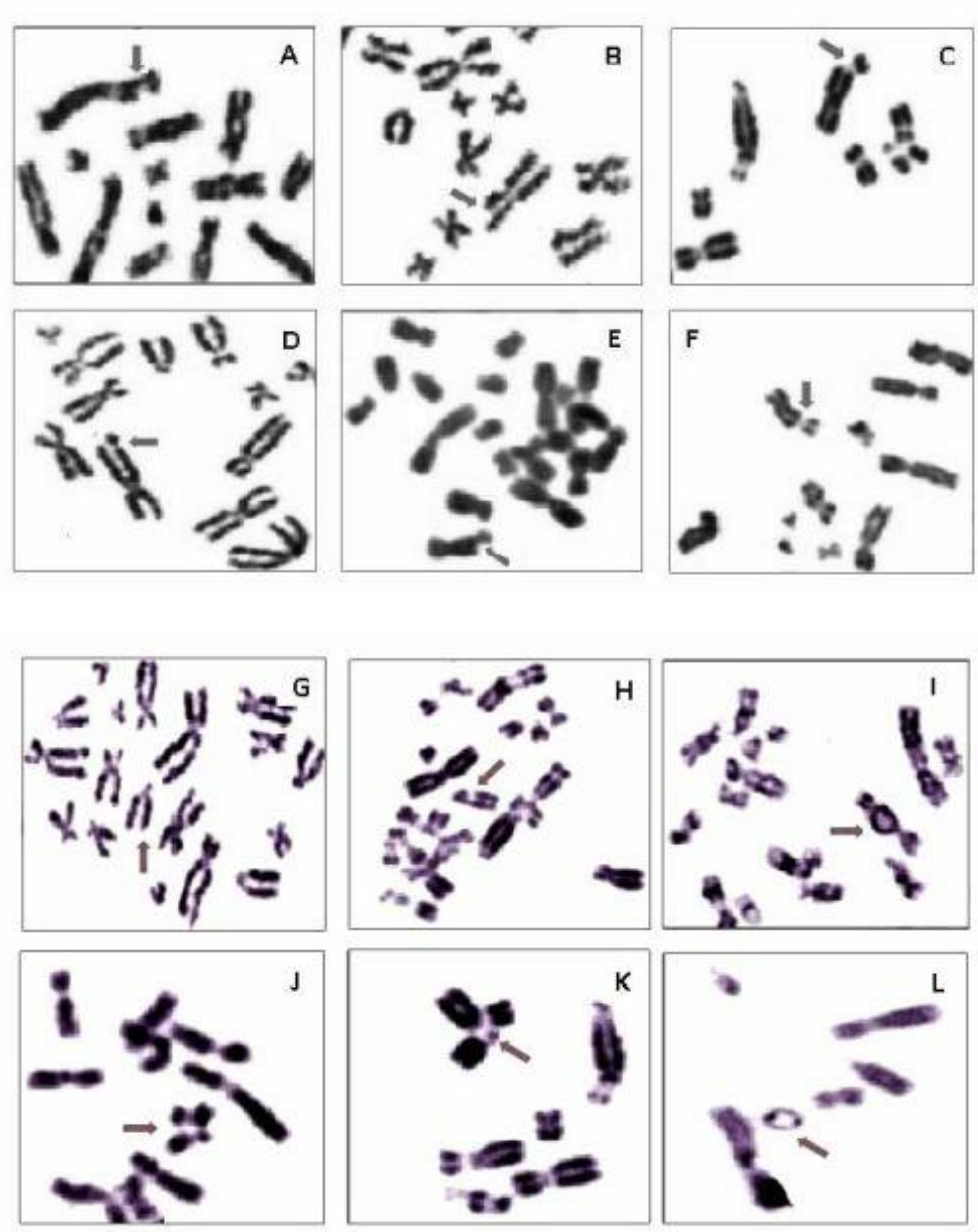

Figure 1: showing A-1p32break B-1p32loss C- 2q3 break D-2q33break E- 6q22break F-Chromosome break , G-14q31 loss H-14q21 break I- Dicentric J-Quadriradial configuration K- Triradial configuration L- Ring chromosome.

Discussion

In the present study, twenty two cases out of forty inves-tigated showed chromosomal fragile sites affecting A to D groups. Among the cases investigated , 19 had CVS abnormalities followed by 16 with respiratory tract in-volvement.

Comparing the systems affected, respiratory system was more affected in the presence of fragile sites which is manifested in the form of frequent infections. Although 68.4% of cases with cardiovascular involvement showed fragile sites in the present study, there was no appearance of secondary constrictions affecting the 2q11 region as quoted by lejune et al [4] in the present series. This is of-ten associated with typical or atypical phenotypic features affecting cardiovascular malformations, growth retarda-tion and mental retardation. Instead, 2p22, 2q32, 2q33 was observed in below 12 months age group in our series [5].

Growth parameters such as height, weight, head circum-ference were compared with fragile sites. Among these only height of the child with fragile sites was significantly decreased when compared to DS children without fragile sites. However, this could not be compared as there was no relevant studies earlier done.

Comparing the instances of fragile sites – involving vari-ous forms/group of chromosomes/ bands in cases with various degrees of mental retardation observed in the cur-rent study. Only twenty two cases had Chromosomal aberrations. The remaining 18 were karyotypically of normal picture of Trisomy 21. The degree of retardation was classified as severe, moderate and mild with degree of DQ ranging from <34, 35-49 and 50-69 respectively.

In a group where the mental retardation was severe – DQ (<34), the chromosomes affected were 1q32, 1p32, 2q22, 2q33, 2p22, 4q25, 14q21 and 14q3. The aberrations were mainly of chromosomal breaks, Chromosomal loss, trira-dial and quadriradial configuration including dicentrics.

In moderate retardation group – DQ (35-49), the chromo-somes affected were 1p32, 3q26, 6p21, 4p12. The aberra-tions were chromosomal breaks, triradial and quadriradial configurations including ring chromosomes. While in mild group – DQ (51-69), the chromosomes affected were 3q21, 5q32. The aberrations were chromosomal breaks, triradial and quadriradial configurations. There were no reports regarding the association of degree of mental re-tardation with chromosomal aberrations in Trisomy 21.The classical fragile sites with mental retardation was described in detail only for X chromosomal complement; wherein, FRAXA, FRAXE and FRAXF have been iden-tified at Xq28 in fragile X syndrome.

However, Saxena et al observed 12% of chromosomal aberrations per cell with a low IQ with a higher incidence of common fragile sites affecting 3p14, Xq21.3 in cases with Down syndrome . This was not observed in the cur-rent series ,he stated that the increase in the fragile sites in Down syndrome could be due to nutritional factors such as reduced intake or deficiency of folic acid [6]. Since folic acid levels were not estimated in the present series, fragile sites could not be due to folic acid alone, but pres-ences of fragile sites lead to poor growth and develop-ment and involvement of systemic diseases.

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