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Chromosomal Abnormalities among Children with Congenital Malformations

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KEYWORDS Congenital Malformations. Chromosomal Abnormalities. Chromosomal Variants. Karyotypic Anomalies. Chromosomal Syndromes

ABSTRACT Increase in chromosomal abnormalities is reported in patients referred with birth defects and infertility. This study was aimed to carry out chromosomal analysis for the presence of cytogenetic abnormalities among congenitally malformed children. The karyotypic status could be determined in 176 cases of the 195 malformed children studied. Thirty (about 17%) children exhibited chromosomal anomalies. Among 85 cases with multiple system malformations, 32 (37.6%) showed chromosomal abnormalities and all of them belonged to the category of known syndromes. Chromosomal variants were observed in two children. Down syndrome was the most common syndrome encountered. Evaluation of chromosomal abnormalities is important in understanding the etiology of congenital malformations. Further, a correlation does exist between phenotypic features and the karyotype. Variants are in general, not related directly to the phenotype.

INTRODUCTION

An increased awareness of the occurrence of cytogenetic abnormalities in individuals reporting infertility, mental retardation and/or dysmorphism has increased the demand for chromosomal analysis (Al Husain and Zaki 1999). Several studies have documented an increase in chromosomal anomalies in newborn infants as well as in children referred for cytogenetic evaluation (Milia et al. 1984; Karukaya 1990; Panich and Jinorise 1991; Kenue et al. 1995; Jung et al. 1999). The aim of this study was to determine the frequency of chromosomal abnormalities among children with congenital malformations referred from various hospitals in the city. Further, an analysis of the frequency of system wise malformations and chromosomal abnormalities in the different categories would be helpful in the establishment of phenotype-karyotype correlation, if any. Thus, the study would stress the value of cytogenetic investigation in children having abnormal clinical features.

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MATERIALS AND METHODS

One hundred and three children (about 53%) of the total 195 cases referred had single system malformations. Among the 92 children with multiple congenital malformations, 52 (about 27% of the total) were diagnosed to have known syndromes and the rest 40 had unknown syndromes (Table 1).

The study comprised of 195 children with congenital malformations referred for cytogenetic analysis from different sources during the period September 1994 to November 1999. Patients were referred from four major hospitals within the city and by private practitioners. A questionnaire was designed to document information on the clinical features recorded by the respective pediatrician or neonatologist. Data on prenatal, maternal obs-tetric and family history were obtained through personal interview of the parents accompanying the patients. Clinical photographs were taken when permitted.

The age of the children ranged from one day to 14 years. All the referred patients were broadly classified into two groups based on malformation(s), namely single system malformations and multiple system malformations. Multiple system malformations were further sub-classified into known syndromes and unknown syndromes. Known syndromes were those which were well established (WHO 1989) while the latter were a constellation of malformations that could not be assigned to any previously known syndrome.

Peripheral blood samples were used to obtain chromosomal preparations for analysis. Whole blood cultures were set up employing a modified protocol (Hungerford 1965) Subsequently slides were prepared and GTG- banded (Seabright 1971) for analysis.

Twenty-five well-spread and well-banded metaphases from each individual were analyzed through oil immersion (100x). Chromosomal abnormalities were designated according to standard nomenclature (Mitelman 1995)

Well-banded metaphases were photographed using Nikon photomicroscope (Labophot–2). NOVA (125 ASA) black and white film and Kodak (125 ASA) colour film were used to photograph banded metaphases and clinical features of patients respectively.

RESULTS

The karyotype could be determined in 176 cases of the 195 malformed children subjected to chromosomal analysis. Thirty two (about 18%) children exhibited anomalies which included two chromosomal variants. All 91 children who exhibited single system malformations showed normal karyotype. Among 85 cases with multiple system malformations, 32 (about 38%) showed chromosomal abnormalities and all of them belonged to the category of known syndromes (Table 1). Twenty-nine children were referred with clinical features of Down syndrome (DS), of whom twenty-seven showed an abnormal karyotype (Table 2). Twenty-five DS children exhibited trisomy for chromosome 21 of whom 16 were males and 9 were females (Fig.1A). One was a translocation Down carrying a Robertsonian translocation between chromosomes 14 and 21 (Fig.1B) and the other was a mosaic with a normal cell line besides the trisomic cell line. The proportion of trisomic cells in the latter was 64%. All the parents showed a normal karyotype.

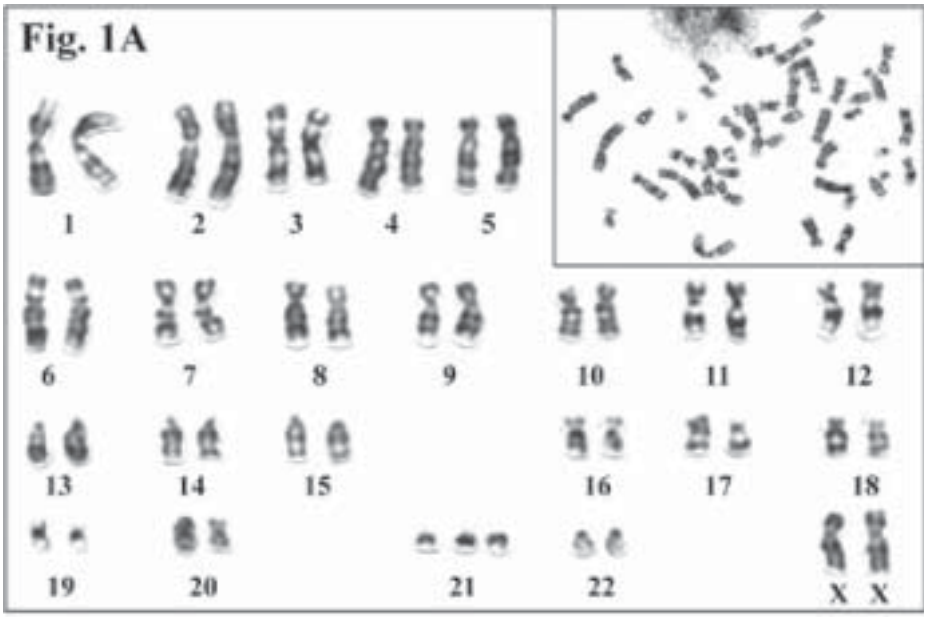
Of the three patients who showed clinical features typical of Turner syndrome (Fig. 2A), only one showed a typical Turner karyotype with 45,X chromosomal pattern (Fig. 2B) besides a normal cell line. The clinical features were short stature, short and webbed neck, low hair line, high arched palate, broad chest, widely placed nipples and pulmonary valve regurgitation. The only case of Edward syndrome with characteristic clinical features like low set ears, small chin, prominent occiput, clenched hands with overlapping index and fifth fingers, single palmar crease, rocker bottom feet, and ventricular septal defect (Fig. 3A) was found to be trisomic for chromosome 18 (Fig. 3B). A boy aged 10 years with Cri-du-chat syndrome (Fig. 4A) exhibiting short stature, microcephaly, downward slant of eyes, facial asymmetry, mental retardation along with a jolly appearance showed a terminal deletion at band 5p14 (Fig. 4B) (Table 2).

Chromosomal variants were observed in two children. The first was a boy with Ochoa syndrome with urofacial abnormalities, who possessed a variant chromosome 14 showing a prominent satellite (Fig. 5A). The father of the boy who is also a carrier for this chromosomal

Table 1: Frequency of chromosomal abnormalities and variants in children with congenital malformations

System	*N*	*n*	*Chromosomal abnormalities / variants*	
			No.	*%*
Single System Malformations	103	91	0	0.0
Multiple Congenital Malformations	92	85	32	37.6
A. Known Syndromes	52	47	32	68.1
i. Down syndrome	29	29	27	93.1
ii. Other than Down syndrome	23	18	5	27.7
B. Unknown Syndromes	40	38	0	0.0
Total	195	176	32	18.2

N = number of malformed children referred
n = number of malformed children successfully karyotyped



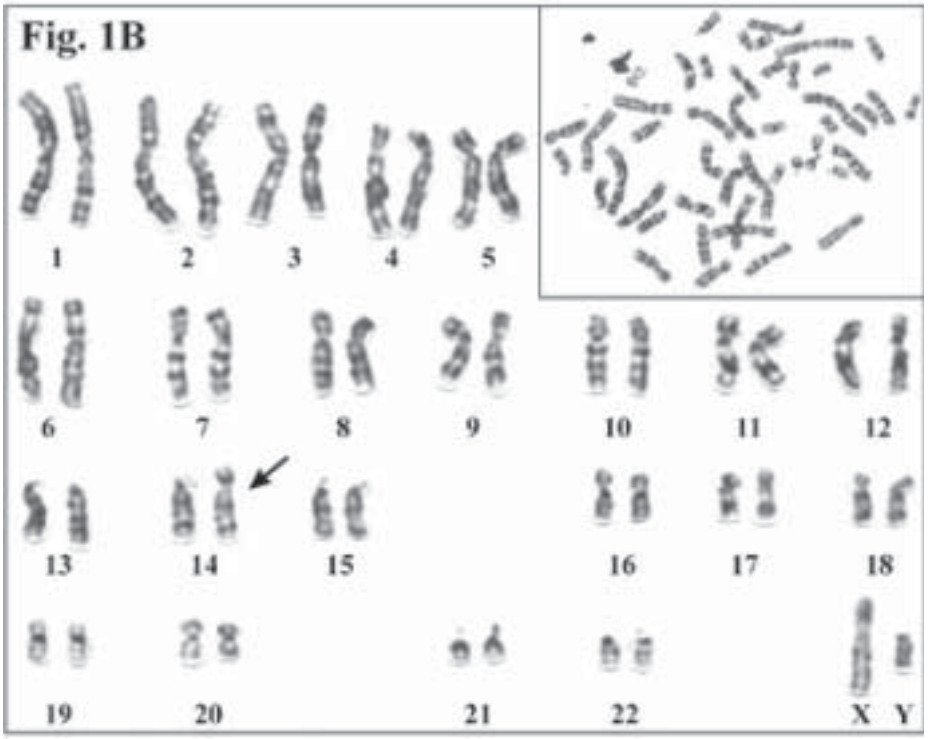


Fig. 1A. GTG-banded karyotype of female baby with Down syndrome: 47,XX,+21
Fig. 1B. GTG-banded karyotype of male baby with Down syndrome showing Robertsonian translo-cation: 46,xy,der(14;21)(q10;q10),+21

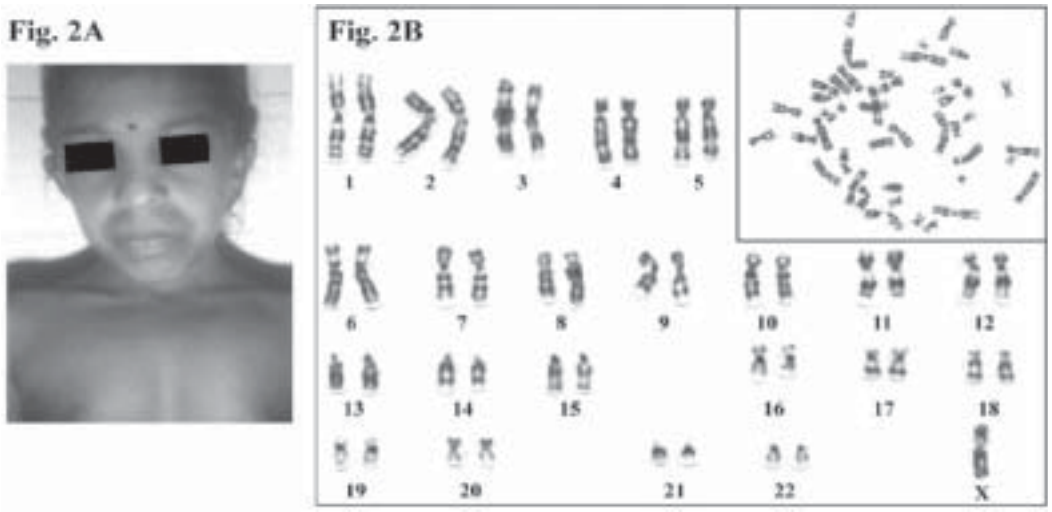


Fig. 2A. Photograph of Turner child showing a short neck with a webbed appearance and low set ears.
Fig. 2B. GTG-banded karyotype of female child with Turner syndrome: 45,X

Table 2: Cytogenetic data in children with congenital malformations

System	*N*	*n*	*Karyotype*	*Karyotype of carrier parent(s)*
Single System Malformations	91	-	46,XX (36) 46,XY (55)	
Multiple Congenital Malformations	85			
A. Known Syndromes	47	32		
i. Down syndrome	29	16	47,XY+21	Normal
		9	47,XX+21	Normal
		1	46,XY,der(14;21)(q10;q10),+21	Normal
		1	47,XX,+21/46,XX	Normal
ii. Other than Down syndrome	18	1	45,X/46,XX	Normal
		1	47,XX,+18	Normal
		1	46,XY,del(5)(p14)	Normal
		1**	46,XY,14ps+pat	46,XY,14ps+
		1**	46,X,inv(Y)(p11q12)	46,X,inv(Y)(p11q12)
B. Unknown Syndromes	*38*		46,XX (20) 46,XY (18)	
Total (I + II)	176	32 18.20%		

N = number of malformed children successfully karyotyped
n = number of children with chromosomal abnormalities / variants;
** Chromosomal variants

variant did not show any phenotypic abnormality. The other variant was a pericentric inversion involving the distal heterochromatic region of Y chromosome (Fig. 5B) which was observed in a child with Zellweger syndrome having phenotypic abnormalities like high forehead and pear shaped skull, hypertelorism, high arched palate, hypotonia and single palmar crease. This inversion was inherited from his father who however, was phenotypically normal.

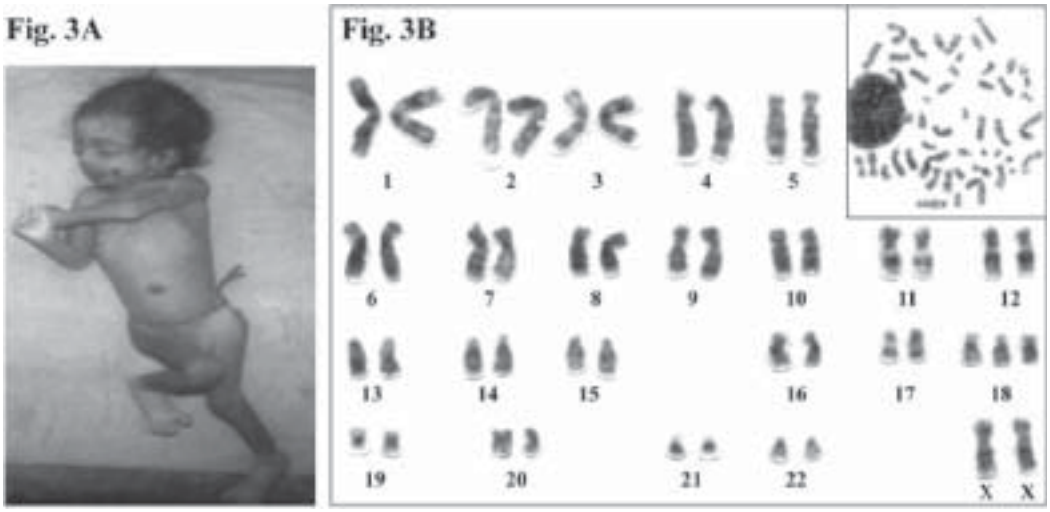


Fig. 3A. Photograph of female baby with Edward syndrome showing low set ears, rocker bottom feet and overlapping fingers
Fig. 3B. GTG-banded karyotype of the female baby with Edward syndrome: 47,XX,+18

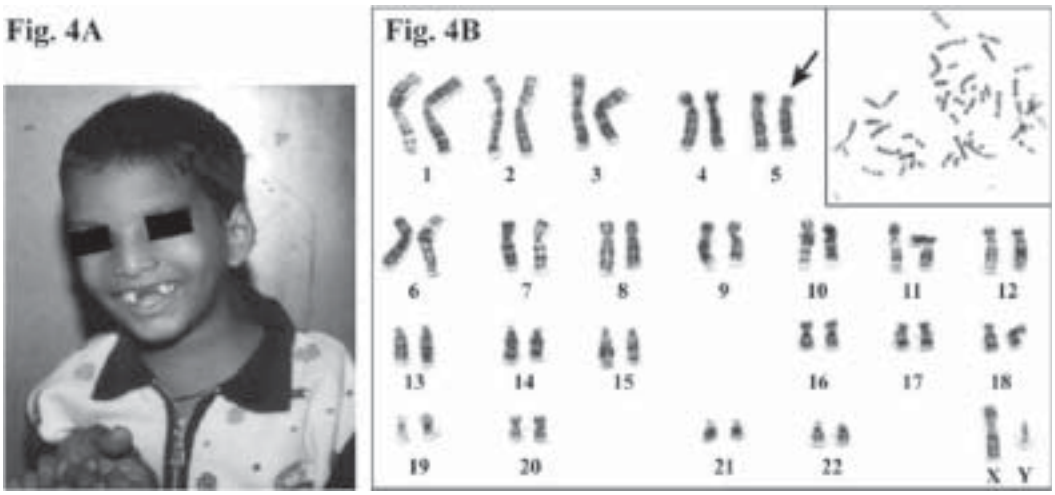


Fig. 4A. Photograph of the boy with Cri-du-chat syndrome showing microcephaly, small asymmetric face, hypertelorism, low set ears and malocclusion of teeth.
Fig. 4B. GTG-banded karyotype of the male child with Cri-du-chat syndrome: 46,XY,del(5)(p14)

DISCUSSION

Single system malformations were found to account for almost 53% of the total children studied. This finding supports an earlier observation that isolated malformations account for 50% of the congenital anomalies (Winter 1996). Down syndrome constituted the most common syndrome, which is in accordance with previous reports (Navsaria et al. 1993; Kenue et al. 1995; Al Husain and Zaki 1999).

About seventeen percent of the children referred with malformations had chromosomal abnormalities, excluding the chromosomal variants. This is in agreement with earlier studies reporting chromosomal abnormalities in 14.3%

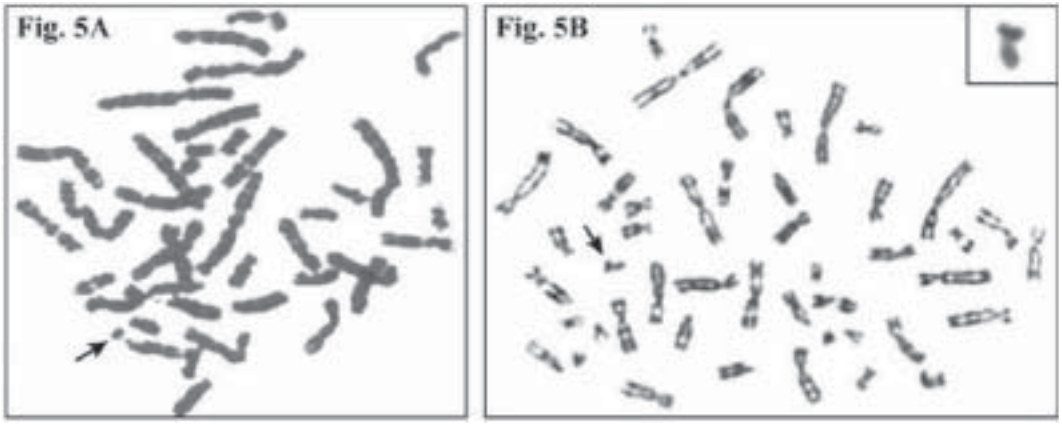


Fig. 5A. GTG-banded partial metaphase of boy with Ochoa syndrome showing a variant chromosome 14 (shown by arrow)
Fig. 5B. GTG-banded metaphase of the boy with Zellweger syndrome: 46,X,inv(Y)(p11q12). Insert shows the inv(Y).

to 18% among the referred cases (Navsaria et al. 1993; Butler and Hamill 1995; Jung et al. 1999). However, higher frequencies of about 21% to 27% of chromosomal abnormalities have been observed in selected populations by other investigators (Milia et al. 1984; Panich and Jinorose 1991) and it could be due to the different criteria employed in the selection of patients. An increased rate of chromosomal abnormalities was observed in a selected population with clinical abnormalities in comparison to an unselected population (Milia et al. 1984).

None of the 91 children with single system malformations showed any chromosomal abnormality. Anomalies involving a single system have been rarely reported to carry sporadic chromosomal abnormalities. Further, most of the isolated malformations involving cardiac, nervous and genito-urinary systems are known to have multifactorial etiology (Mueller and Young 1995).

Trisomy 21 was the most common autosomal trisomy observed in this study. Similarly, various investigators have recorded a high frequency of trisomy 21 in a referred population and the frequency varied from 5.7% to 26.2% (Navsaria et al. 1993; Kenue et al. 1995; Al Husain and Zaki 1999). Of 29 cases diagnosed to have Down syndrome, 27 (93.1%) showed chromosomal abnormalities - 25 exhibited trisomy 21, one had a Robertsonian translocation der(14; 21)(q10; q10) and one was a mosaic - while two possessed a normal karyotype. Previous studies have reported a similar frequency of chromosomal abnormalities ranging between 82.6% to 97.6% among patients with DS phenotype referred for cytogenetic evaluation (Al Husain and Zaki 1999; Jung et al. 1999). A study of DS patients with an apparently normal karyotype but harboring small amount of extra material from chromosome 21, employing molecular methods narrowed the critical region to 21q22.1 to such a degree that is far below the limit of cytogenetic detection (Korenberg et al. 1990).

A single case of trisomy 18 was the other autosomal trisomy registered in this study. Previous investigators have also reported such a low frequency (Navsaria et al. 1993; Al Husain and Zaki 1999). Of the three patients referred with Turner syndrome only one showed the typical karyotypic abnormality, but in addition to the normal cell line. In a recent study, 71% of the Turner syndrome patients referred for cytogenetic analysis had a normal karyotype (Ganguly and Sahni 2003). Cri-du-chat syndrome was encountered in one patient and karyotypic analysis revealed a terminal deletion at 5p14. Various molecular studies have mapped the break points from distal 5p12.2 to 5p15.3 and significance of 5p14 deletion has been debated. It is pertinent to note that the genotype-phenotype correlation observed in our patient was comparable to other patients (Mainardi et al. 2001).

A boy with Ochoa syndrome showed a variant chromosome 14 that was paternally inherited. A normal karyotype was shown in an earlier report (Teebi et al. 1989). One patient with

Zellweger syndrome showed an inversion of the Yqh region inherited from his father who had a normal phenotype. A variety of structural aberrations involving the heterochromatic segment of the Y chromosome without any accompanying phenotypic effects have been reported (Sumner 1990).

CONCLUSION

In conclusion, chromosomal abnormalities are an important cause of congenital malformations, emphasizing the need for cytogenetic evaluation. Further, a correlation does exist between the phenotypic features and the abnormal karyotype. However, few cases with a clinical suspicion of certain syndromes like Down syndrome and Turner syndrome exhibited normal karyotype. Molecular studies could have thrown light on the precise genetic constitution of those patients. Variants are in general, not related directly to the phenotype.

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