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RESEARCH ARTICLE

GENETIC ASPECTS OF CONGENITAL HEART DISEASE IN DOWN SYNDROME

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ABSTRACT

Congenital heart disease (CHD) is a major factor in increased mortality in infancy and childhood. The aim of this study was to determine the frequency of chromosomal abnormalities in Down syndrome (DS) children is important in understanding the etiology of congenital malformations. Down syndrome also called Trisomy 21, is a condition in which extra genetic material causes delays in the way a child develops, both mentally and physically and also is a major cause of congenital heart disease and the most frequent known cause of atrioventricular septal defects (AVSD). Twenty four cases of Down's syndrome were found among 160 children with congenital heart disease under the age of 1day to 14 years, of which 14(58.3%) were males and 10(41.6%) were females, while the remaining 26(16.2%) cases had normal karyotype, Among 74(46.2%) cases with multiple system malformations and 36(22.5%) cases with known and unknown congenital defects. Consanguinity was observed in normal karyotype 30(75%) and abnormal karyotype 10(25%) results in CHD patients. Down syndrome is usually identified soon after birth by a characteristic pattern of dysmorphic features. The diagnosis is confirmed by karyotype analysis and also this study was undertaken to obtain more information about the nature of heart defects associated with Down's syndrome.

INTRODUCTION

Congenital heart defects are the most common of all birth defects, which is found to affect nearly 1% of newborns, and their frequency in spontaneously aborted pregnancies is estimated to be tenfold higher (Behrman *et al*., 2000). The causes for CHD can be categorized into three major groups such as, chromosomal, single gene disorders (10- 15%) and multiple factors (85-90%) (Payne *et al*., 1995). Congenital anomalies having a chromosomal cause, besides causing gross phenotypic anomalies also remain the leading cause of mental retardation (Pashayan *et al*., 1973). So far, more than 100 chromosomal disorders have been reported however, trisomy 21 remains the commonest with its incidence 1:650-1:1000 live births (Hassold and Sherman 2000). The clinical syndrome called Down's syndrome is due to three copies of chromosome 21 instead of only two in all or most of the cells of the body. The extra copy usually arises during the formation of the parental germ cell when two 21 chromosomes stick together in one cell instead of moving apart and taking a place individually in separate cells. This is called non-disjunction. The non-disjunction causing trisomy 21 is of maternal origin in 88% of cases, paternal in 9%, and mitotic in 3 % (Sherman *et al*., 1994). About 75% of maternally derived trisomy 21 is due to non-disjunction at the first meiotic division in the female embryo, though recent work has suggested that non-disjunction occurring in the second meiotic division may actually be initiated in the first (Lame *et al*., 1997).

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The actual gene or genes on chromosome 21 that are responsible for Down syndrome are now being identified in a critical region of 20-40 genes (Epstein, 1995). The clinical manifestations of Down syndrome are numerous and can present in any body system. The most significant include intellectual impairment, short stature, heart disease, digestive disorders and orthopaedic abnormalities. Heart disease is, without a doubt, the main factor contributing to a favourable or unfavourable course in these patients. Among all cases of congenital heart disease, 4%-10% are associated with Down syndrome, and 40%-60% of Down syndrome patients present congenital heart disease. Cardiac malformation is the principal cause of mortality in the first two years of life (Rodriguez, 1984; Stoll *et al*., 1998). Trisomy 21 causes Down syndrome, the human congenital anomaly of special interest. Its association with congenital heart disease is well known, the reported incidence being 40%-60%. (Shaher *et al*., 1972). This congenital heart disease contributes significantly to the morbidity and mortality of children with Down syndrome, who may develop congestive heart failure, pulmonary vascular disease, failure to thrive or pneumonia. Atrioventricular canal defect, is the most serious of the defects. It is a severe heart defect with specific clinical features. In the normal formation of the heart the endocardial cushions grow toward each other and leave openings between the atria and the ventricles and followed by Patent Ductus Arteriosus (PDA) and Atrial Septal Defect (ASD). Other forms of complex heart disease can occur including overriding aorta and tetralogy of Fallot (Berr and Borghi, 1990). A high frequency of congenital heart disease occurs in children with Down syndrome from a population

with widely prevalent consanguinity (Becker *et al*., 2001). Overall, the incidence of all congenital anomalies is increased two fold in children with Down syndrome. DS is one of the most intensely studied genetic syndromes because of its frequency in our population and its medical significance. This study was served to explore the relation between congenital anomalies with some form of chromosomal abnormality, the majority of which are Down syndrome.

MATERIALS AND METHODS

Material

The present study included a total of 160 patients with congenital heart disease. The age of the children ranged from one day to 14 years. We collected information of the presence of categoric congenital risk factors in every patient. Based on the cytogenetic findings, the patients and their families have been appropriately counseled and the possible management options were explained to them.

Collection of Samples

For the cytogenetic analysis the blood samples were processed employing routine lymphocyte cultures. About 2-5ml of venous blood was collected from the children in sterile disposable syringe containing heparin and was mixed well by tilting the syringe, 2-3 times. Heparin prevents coagulation without diminishing the quality of the preparation. Peripheral blood is a plasma-based suspension of erythrocytes, platelets and leukocytes; only the leukocytes are nucleated and therefore divide.

Culture

The lymphocytes were stimulated by the use of mitogen. The mitogen transforms the lymphocytes into mitotocally active cells. PHA (Phytohaemagglutinin) a mucoprotein was added to the lymphocytes in the culture. The constituents of the lymphocyte culture include RPMI 1640 basal medium, supplements such as serum (usually fetal bovine serum) that contains various growth factors essential for cellular proliferation. Antibiotics were added to control/ prevent the microbial infection. PHA was added to stimulate lymphocyte proliferation.

Harvesting Lymphocyte Culture

After 72 h of incubation of the lymphocyte culture, it was harvested by first adding colchicine, which arrests the dividing cells in the metaphase, when the chromosomes are maximally condensed and easiest and are easy for analysis. After exposure to colcemid, the cells are subjected to a hypotonic solution (0.075M). This solution causes swelling of cells, rupture of nucleus and better separation of the individual chromosomes. Centrifugation of the sample results in supernatant and the pellet is seen at the bottom of the test tube. The supernatant is removed and the pellet that contains the lymphocytes is "fixed". "Fixation" of the cell pellet is a process whereby the cells are killed, nuclear proteins removed and the chromosomal morphology is preserved. The fixative consists of 3 parts of methanol and one part of acetic acid. After fixation the pellet is dropped from a height onto a labeled slide and allowed to dry. These slides were later stained using Giemsa stain (GTG banding).

Staining of Slides

The slides were aged for 3-4 days at room temperature and then stained. These slides were first dipped in trypsin and subsequently stained with Giemsa. The banding pattern reflects both structural and functional components of the chromosomes. Dark bands are AT rich regions containing few active genes as compared to the light bands.

Chromosomal Analysis

Chromosomes spreads are analysed under the microscope. The banding pattern helped to analyse the structural variations and the numerical variations. Fifty metaphase spreads were counted, 5 drawn, 3 spreads captured using CCD camera and printed, one of these prints was chosen for karyotyping and results were recorded. Karyotyping is a display of the chromosomes according to their length and position of the centromere of the chromosome (metacentric, submetacentric, and acrocentric). Thus, cytogenetic abnormalities, were detected, interpreted and results were correlated with the clinical presentations, in order to indicate their implications.

RESULTS

The most common CHD associated with DS worldwide is found to be Atrioventricular canal defect, followed by Ventricular septal defect , Atrial septal defect, Coarctation of Aorta (COA), Tetralogy of Fallot (TOF), Patent ductus arteriosus (PDA). In the present study, among the numerical chromosomal abnormalities, free Trisomy 21 was found in 24 CHD patients. In general, all of the recent reports listed in Table 1 confirmed that an AVSD is the most common heart defect in DS 56 (35%),ventricular septal defect 48 (30%), ASD 14 (8.7%), TOF 8 (5%), PDA 18 (11.2) and other heart defects 20 (12.5) of DS infants, the latter proportion being from the present study. Although only the more recent studies of CHD in DS are discussed here and emphasis is placed on the importance of modern methods of cardiac diagnosis and associate with several clinical characteristic craniofacial and physical features of DS, identified cytogenetically. The common clinical features found in these patients were CHD, mongoloid slant and flat nasal bridge (Table 2).

The cytogenetic karyotyping analysis could be determined in 160 CHD cases of the 24 DS defects children subjected to chromosomal analysis. All 86 (53.7) children who exhibited single system malformations showed normal karyotype. Among 74 (46.25) cases with multiple system malformations, 18 (about 11.2%) showed chromosomal abnormalities and all of them belonged to the category of known syndromes. Twenty-four (13.1) children were referred with clinical features of Down syndrome, of whom 4 (2.5) showed an other than Down syndrome abnormal karyotype (Table 3). Most of the parent's results showed a normal karyotype and some of them have Consanguinity and increased maternal age. 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 (Figure 2).

Table 1. Congenital heart diseases in Down syndrome

CHD	Number (%)
AVSD	Total 56 (35%)
Complete Isolated	20
With 2° ASD only	7
And arch abnormality[a]	3
And TOF	3
With persistent PDA only	8
With arch abnormality[b]	4
Partial	
1° ASD	4
Inlet VSD	7
VSD	Total 48 (30%)
Isolated	21
With 2° ASD (±PDA) only	9
With 2° ASD and other[c]	6
With persistent PDA only	7
With other[d]	5
2° ASD (isolated)	14 (8.7%)
TOF (without AVSD)	8 (5%)
PDA (persistent)	18 (11.2%)
Other heart defects[e]	20 (12.5%)
Total	160 (100%)

[a]Aberrant left subclavian artery; [b]Right aortic arch, coarctation; [c]Double outlet right ventricle (2), pulmonic stenosis (2). [d]Coarctation, double aortic arch, and pulmonic stenosis.
[e]Vascular ring (aberrant left subclavian artery and right aortic arch).

Table 2. Characteristic clinical features of congenital heart disease patients with Trisomy 21

S.No	Characteristics features	Clinical features observed	
		No	Expression in %
1	Congenital heart diseases	16	66.6
2	Mongoloid slant	7	29.1
3	Simian crease	4	16.6
4	Hypotonia	10	41.6
5	Epicanthal folds	5	20.8
6	Microcephaly	6	25.0
7	Flat nasal bridge	4	16.6
8	Brachycephaly	3	12.5
9	Delayed development	7	29.1
10	Brachydactyly	4	16.6
11	Ear abnormalities	5	20.8
12	Short stature	3	12.5
13	Brushfield spots	0	0.0

Our study was to ascertain the relation of consanguinity to Down syndrome (DS), and pattern of congenital heart defects in normal karyotype 30 (75%) and abnormal karyotype10 (25%). The non consanguinity results showing in normal karyotype100 (83.3%) and abnormal karyotype 10 (16.6%). The results were analyzed and compared with 40 consanguinity and 120 non consanguinity DS syndrome patients, and pattern of CHD. DS children who are products of consanguineous marriage have severe forms of CHD, and frequency of CHD is more among male and then female DS children. Detailed results are shown in following Table 5.

Table 4. Relation between maternal age and birth of Down syndrome.

S.No	Maternal age	Number of birth	Percentage
1	20 - 25	04	16.6
2	26 - 30	06	25.0
3	31- 35	10	41.6
4	36 - 40	04	16.6
	Total	24	99.2

Table 5. Consanguinity in relation to Down syndrome

	Normal karyotype	Abnormal karyotype	Total
Consanguinity	30(75%)	10(25%)	40
Non Consanguinity	100(83.3%)	20(16.6%)	120
Total	130(81.2%)	30(18.7%)	160

DISCUSSION

Trisomy 21 can affect many aspects of development, producing a wide and variable set of clinical features (Cohen, 1999). This aneuploidy is the leading cause of congenital heart disease and the most frequent genetic cause of mental retardation. A high frequency of congenital heart disease occurs in children with down syndrome from a population with widely prevalent consanguinity (Teebi and Feraag, 1997). dlthough down's syndrome is usually linked to intellectual disability, the morbidity from other associated congenital

Table 3. Cytogenetic data in children with congenital malformation

System	Number (%)	n (%)	Karyotype	Karyotype of carrier parents
Single System Malformations	86(53.7)	45(52.3)	46,XY	
Multiple Congenital Malformations		41(47.6)	46,XY	Normal
A. Known Syndromes				
i. Down syndrome	18(11.2)			46,XY,der(14;21)
	24(13.1)	14(58.3)	47,XY+21	Consanguinity/Increased
ii. Other than Down syndrome	4(2.5)	10(41.6)	47,XX+21	maternal age
				45,X/46,XX
B. Unknown Syndromes	28(17.5)		46,XY	
			46,XY	Normal

There is a pronounced maternal-age effect on the occurrence of trisomy 21, with increased risk when maternal age passes 35 years. Down syndrome is associated with maternal ages at the extremes of the childbearing period. In the present study, majority of women belonged to the age group of 20 – 25 (16.6%), 26 – 30 (25%), 31-35 (41.6%) and 36 – 40 (16.6%). The least production of the children was observed in advanced age group (Table 4).

abnormalities is considerable. The three most common types of congenital heart defects reported in the two registry studies were atrioventricular canal defects (28% of those with heart defects in the Dublin study and 43% in the Strasbourg study), ventricular septal defects (28% and 32% respectively), and patent ductus arteriosus (21% and 5% respectively). Other studies report similar findings. (Tubman *et al.,* 1991). The trisomy 21 was the most common autosomal syndrome and

Figure1: Clinical feautures of down syndrome

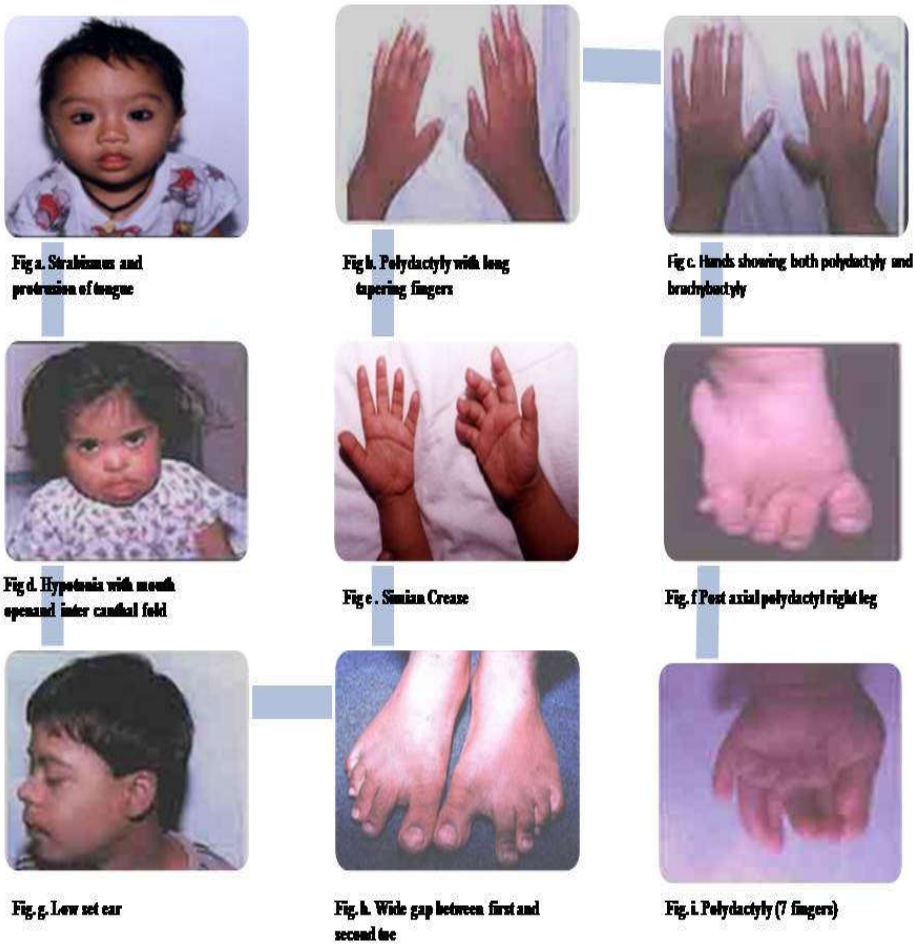


Fig a. Strabismus and protrusion of tongue

Fig b. Polydactyly with long tapering fingers

Fig c. Hands showing both polydactyly and brachydactyly

Fig d. Hypotonia with mouth open and inter canthal fold

Fig e. Simian Crease

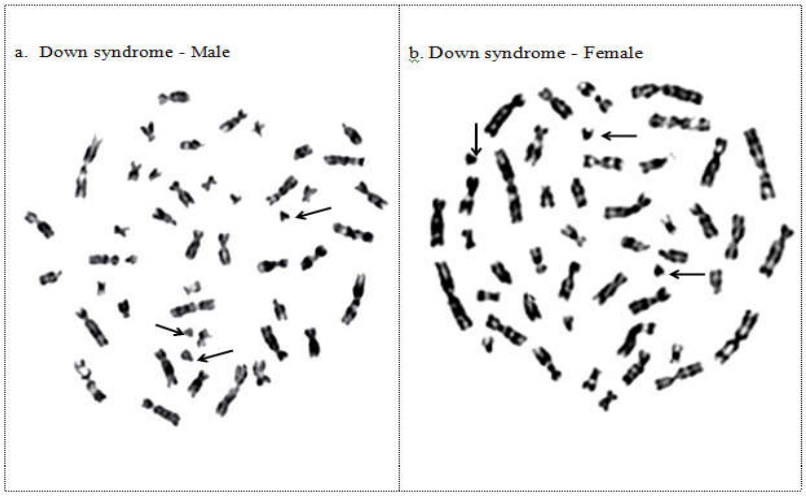
Fig. f Post axial polydactyl right leg

Fig. g. Low set ear

Fig. h. Wide gap between first and second toe

Fig. i. Polydactyly (7 fingers)

Figure 2. Karyotyping analysis of G-banded chromosomes of the CHD in Down syndrome



associate with congenial heart disease results was experimentally observed in the cytogenetic analysis of this study 24(13.1%), 47, XY+21; 47, XX+21. The atrioventricular canal defect is the most serious of the defects. It is a severe heart defect with specific clinical features. In the normal formation of the heart the endocardial cushions grow toward each other and leave openings between the atria and the ventricles. In the Dublin study, among children born between

1980 and 1989 in which follow up was completed to 1992, 26% of those affected with atrioventricular canal defects had cardiac surgery. Of these, 88% survived compared with 47% survival in those who did not have cardiac surgery. Of all the heart defects only atrioventricular canal defects had an adverse impact on survival, with 68% of those affected surviving to 1 year and 58% to 10 years compared with 93% and 90% respectively for those without this defect. Atrioventricular canal defects are genetically linked to chromosome 21 (Burn and Goodship, 1996) and are about 420 times more common in those affected with Down's syndrome than in the general population (Connor and Ferguson-Smith, 1996). In the present study results was showed that the association between congenital heart disease and Down syndrome in atrioventricular septal defect 56 (35%), ventricular septal defect 48 (30%), ASD 14 (8.7%), TOF 8(5%), PDA 18(11.2) and other heart defects 20(12.5). It has long been known that common atrioventricular canal and ventricular septal defect are common cardiac malformations in patients with Down's syndrome.

The Down syndrome birth has often been associated with maternal age by various workers (Waheid *et al*., 2008a). As reported by the workers, increased maternal age has generally been associated relationship with non disjunction of chromosome number 21.It is estimated that 80% of Down syndromes are born to woman <35 years, however, in the present study only 6 (8.4%) females were in the age group >35 years. Therefore, in the present study, majority of the mothers were <35 years. Trisomy 21 could be the consequence of non-disjunction that might occur during gametogenesis or in the 1st or 2nd cleavage (Pullian and Huether 1986; Wright, 1990). Nondisjunction could occur at any time; therefore children with trisomy 21 can be born to mothers of all age groups. Since in the present study majority of mothers are <35 years, it may therefore, be attributed to the fact that most pregnancies occur in younger woman. The maternal age of CHD in Down syndrome patients results was showed 20 – 25(16.6%), 26 – 30(25%), 31- 35(41.6%) and 36 – 40(16.6%). Hence, the present findings on the association of maternal age with the birth of Down syndrome are similar to the earlier reports.

The role of consanguinity in congenital malformations like DS and CHD has been studied by several authors from high consanguinity rate of many countries (Wahab *et al*, 2006). The risk for birth defects in the offspring of first cousin matings has been increased to 5-8% compared to 2- 3% in non-consanguineous marriages (Magnus, 1985). The prevalence of consanguinity reported in India is 36% and uncle-niece and first cousin are the more commonly occurring relationships in Indian population (Nath, 2004). This important feature of our study implicates probable contributive effect of consanguinity for the occurrence and severity of CHD in DS results showing normal Karyotype 30 (75%) and abnormal Karyotype 10 (25%).The non consanguinity results showing in normal karyotype100 (83.3%) and abnormal karyotype 20 (16.6%) (Table 5). Our study showed VSD as most common lesion followed by AV canal defects, atrial septal defect and patent ductus arteriosus. So it seems possible that consanguinity appears to be an associated risk factor for the severity and rate of CHD in DS. Although studies world over has observed an association between the consanguinity and CHD (Husain and and Bunyan, 1997), but simultaneously has discouraged the association of consanguinity and DS (Hammamy *et al*., 2001).

CONCLUSSION

In conclusion approximately 40% of children born with Down syndrome will have CHD. Atrioventricular defects and VSDs are the most common lesions. Newborns with these lesions may be asymptomatic and have no murmur. Therefore, all babies born with Down syndrome should have an early echocardiogram to screen for CHD. Babies with Down syndrome and a large ventricular shunt are at risk of developing pulmonary vascular disease early in life. The 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.

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