DOI: 10.7860/IJARS/2016/23429:2249

Original Article

Anatomy Section

Prevalence of Down's Syndrome in Patients of Congenital Heart Disease in Vidarbha Region Central India

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ABSTRACT

Introduction: Down's syndrome is a genetic state in which a person has 47 chromosomes instead of 46, with an additional copy of chromosome number 21. This additional genetic material disorders the normal growth process leading to medical and physical defects in cases of Down's syndrome. Children with Down's syndrome are distinguished by mental retardation and typical facial features but they also have high occurrence of a variety of Congenital Heart Disease (CHD).

Aim: This study was done with an aim to estimate the occurrences of CHD among the patients with Down's syndrome.

Materials and Methods: In this cross-sectional study, 374 cases of heart disease from age group of 0 to 14 years were studied for the occurrence of Down's syndrome during the period of 2008 to 2012. The standard karyotyping method was used to confirm Down's syndrome. The normal children of equivalent age group were taken as control. The heart defects were also collected. The collected data was compared with the globally accepted statistics of Down's syndrome.

Results: Out of 374 diagnosed cases of CHD, seven cases (four males and three females) were confirmed with the Down's syndrome (1.87%). The Down's syndrome frequency (1.87%) which is significantly very high in comparison with the age matched control patient's frequency of 0.1% (1 in 1000).

Conclusion: CHDs were prevalent in the children with Down's syndrome, whereas the prevalence of CHD was significantly low when compared to controls of same age groups. The frequency of CHD among children with Down's syndrome is found to be 1.87%. The CHD may appear with the birth and are more common in Down's syndrome.

Keywords: Congenital anomalies, Karyotyping, Mental retardation

INTRODUCTION

Down's syndrome is a genetic condition in which a person has 47 chromosomes instead of 46, with an additional replica of chromosome number 21. The British physician named Langdon Down in 1866 describes the syndrome [1]. Children with Down's syndrome are distinguished by mental retardation and typical facial features but they also have high occurrence of a variety of heart defects. The additional genetic material from chromosome 21 disorders the normal growth process leading to medical and physical defects in cases of Down's syndrome like mental retardation and, typical facial features, small head circumference, protruding tongue, small ear and slopping palpebral fissure [2]. The prevalence of Down's syndrome in India is 0.88 per 1000 (1 out of 1139) to 1.09 per 1000 (1 out of 916). Every hour three Down's syndrome children were report to be born [3]. Congenital heart diseases (CHDs) are the most common with incidence of almost 1% of the world population [4]. Inborn deformities of the heart and great vessels are among the most common of the entire congenital anomalies identified in 1st year of life [5] out of which the most common defects are atrioventricular septal defect, ventricular septal defect, Persistent ductus arteriosus and Tetralogy of Fallot. They comprise one of the most important reasons of infant mortality and morbidity during puberty and then in adult life. Extracardiac deformities found in 15-45% of cases with CHD [6]. However, there is a need of population based records on incidence and distinctiveness of CHD in Down's syndrome. In this study of CHD cases from Vidarbha Region, Central India, the primary aim was to estimate the frequency of CHD among patients with Down's syndrome.

MATERIALS AND METHODS

In this cross-sectional study analysed data was collected from different Medical colleges in Vidarbha region. The study was carried out in Genetic Laboratory, Department of Anatomy of NKP Salve Institute of Medical Sciences and Research Centre, Nagpur, India. The ethical clearance obtained from the Institute Ethical Committee (IEC).

Patient Selection

Total 374 cases of CHD cases from different medical colleges in Vidarbha Region, Central India, between the age group of 0 to 14 years were studied for the occurrence of Down's syndrome during the period of 2008 to 2012. The informed consent obtained from patients and their parents. The all other types of heart diseases were excluded from the study.

Data Collection

The common parameters like age, sex and stages of heart disease were collected. The age at the time of diagnosis of heart defects was also recorded. The normal children of equivalent age group were taken as control. The collected data was compared with the estimated statistics of Down's syndrome in Vidarbha region. Parents were also investigated wherever possible.

Sample Collection

The 2 mL peripheral blood samples were collected under sterile conditions by venipuncture into heparinized tubes for cytogenetic investigation from patients.

Cytogenetic Investigation

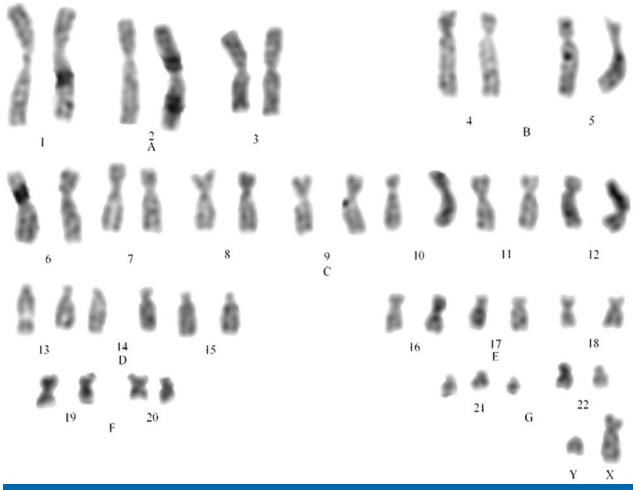
Cytogenetic investigation was carried out on 18 cases of CHD with clinical features similar to Down's syndrome. PHA-stimulated peripheral blood leucocytes were cultured for 72 hours in RPMI-1640 medium supplemented with 20% qualified; heat inactivated fetal bovine serum, 100 U/mL penicillin and streptomycin, without mitogen at 37°C. The culture was exposed to colchicine (10 µg/mL) for 90 minutes followed by hypotonic treatment by potassium chloride (0.075 M KCl) for 20 minutes at 37°C. Then fixed in Methanol: Glacial acetic acid (3:1) and dropped on wet ice cold grease free slides. The chromosomes were G-banded with trypsin-giemsa banding. Olympus BX51 Research microscope was used to screen, capture and karyotype the metaphase chromosomes. The results interpreted according to International Standard Chromosome Nomenclature (ISCN).

STATISTICAL ANALYSIS

The Epi Info® (version 6.0) statistical tool was used for analysis of the result. The Down's syndrome frequency in CHD was compared with normal Down's syndrome frequency, 1 in 1000 with the significant level of $p<0.05$ by using the Pearson's correlation.

RESULTS

During the study period from 2008 to 2012, total 374 children aged from 0-14 years were confirmed diagnosis of non-traumatic, non-acquired CHD. Out of these 18 cases of CHD with clinical features similar to Down's syndrome. The karyotyping process has confirmed seven (four males and three females) of them as Down's syndrome [Table/Fig-1] having the frequency of 1.87 %. In [Table/Fig-2] the characteristics of the cases are summarised. The seven CHD cases associated with Down's syndrome, along with the age at which the patients diagnosed with CHD were noted in the [Table/Fig-2]. Four out of seven diagnosed with the CHD at birth. The value obtained from the Down's syndrome frequency in CHD was significant to the normal Down's syndrome frequency with accepted significant level of $p<0.05$ [Table/Fig-3,4]. After the clinical investigation the four types of CHD were observed among the seven patients that is Endocardial Cushion Defect (ECD),



[Table/Fig-1]: 47, XY Karyotype of the Down syndrome case No. 6.

Case No.	Sex	Age at CHD Diagnosis	Types of Heart Defect
1	F	At birth	ECD
2	M	At birth	VSD
3	F	At birth	ECD
4	M	At birth	ECD
5	F	2 years	ASD
6	M	8 years	VSD
7	F	10 years	PDA

[Table/Fig-2]: CHD cases associated with Down's syndrome.

	Heart Defect Observed	No Heart Defect Observed	Total
Down's Syndrome Cases	7 (87.5%)	1 (12.5%)	8 (100%)
Total Cases Person-Time	381 (27.6%)	1000 (72.4%)	1381 (100%)

[Table/Fig-3]: Statistical analysis of CHDs and Down's syndrome.
*Single table Analysis, Comparing 2 Person-Time Rates at 95% Confidence Level

Test	Value	p-value (1-tail)	p-value (2-tail)
z-score	3.791	0.00007494	0.0001499
Fisher exact		0.0007383	0.001477
Mid-P exact		0.0003859	0.0007718

[Table/Fig-4]: Statistical analysis of Z-score and exact measurement of association between CHDs and Down's syndrome.
*The result is significant $p < 0.05$.

Ventricular Septal Defect (VSD), Patent Ductus Arteriosus (PDA), Atrial Septal Defect (ASD), Out of which we found ECD was most common with three patients, VSD with two patients and ASD, PDA, with one each.

DISCUSSION

It is relatively complex to assess cardiac function in Down's syndrome patients who poorly cooperate due to intellectual disability. At present, limited data is available concerning cardiac function in Down's syndrome cases. CHD refers to a range of inborn heart defects. The defects are coarctation of the aorta, aortic valve stenosis, pulmonary valve stenosis, Ebstein's anomaly, patent ductus arteriosus, septal defects, single ventricle defects, Tetralogy of Fallot, transposition of the great arteries, total anomalous pulmonary venous connection, truncus arteriosus [7]. Out of several studies from the western communities the most common heart defect (about 2/3) found in Down's syndrome are endocardial cushion defect or atrioventricular canal defect [8-9]. In most cases, when a child is born with CHD, there is no well-known cause for disorders. Physician identify that some types of CHD can be linked to child's chromosomes, single gene mutation, or environmental factors. In most cases, there is no particular cause for the heart defect, and they are usually considered to be originated by multifactorial inheritance. In its inclusive form, there is a hole in the septum. In addition, rather than two valves within the heart, there is a large lone valve. This imperfection requires premature surgical repair in early life. In some of the Asian study, a Ventricular Septal Defect (VSD) is the very common defect, where in Latin American study, an Atrial Septal Defect (ASD) is allegedly the most common defect [10,11]. About half of all children with Down's syndrome have CHD [12]. In 90% of cases, a type of septal defect is present in children. Other Numerical chromosomal disorders like Edward syndrome, Turner syndrome and Patau syndrome are associated with CHD [13]. In Schneider [14] study the CHD occurs in 40 - 50% of children with Down syndrome and cardiac abnormalities are probably the most common malformations seen in Down's syndrome.

In most of the study Down's syndrome patients were interviewed and frequency of CHD assayed among them [Table/Fig-5] [15-20], instead of interviewing the Down's syndrome patients we investigate paediatric CHD patient in search of the clinical features similar to Down's syndrome, this retrospectral aspect is unique in the study. The karyotyping results add more significance in the study. In the study we interviewed 374 cases from pediatrics heart disorders investigate clinically for presence of any features similar to Down's syndrome. Out of 18 cases of CHD with clinical features similar to Down's syndrome 7 of them confirmed as Down's syndrome having the frequency of 1.87% [Table/Fig-6] that is significantly very high as compared to the control patients of same age with frequency of 0.1% (1 in 1000).

According to Korenberg et al., [21] CHD seen in Down's syndrome is possibly due to the trisomy of chromosome. In earlier cytogenetic examination the duplications of regions 21 q 22, 0% CHD, was evident. 33% patients had atrioventricular septal defects; his study suggests a single locus responsible for most of the variability of the trait. Genetic variation in COL6A1-COL6A2 gene locus on chromosome 21 was calculated by Davies et al., [22] in 113 controls and 58 European families having a child with trisomy 21. There were statistically major divergences between subgroups of trisomy 21 children with and without CHD [23]. These COL6A1-COL6A2 genes may be responsible for the congenital malformations of the heart and major vessels. All patients with Down's syndrome should have a cardiac assessment at birth due to the high frequency of involvement with heart defects, which increase mortality during the first year of life [24]. In the Vilas Boas et al., study a relevant fact is that most patients had echocardiograms (93.6%), which suggests that an adequate and early cardiac evaluation was performed; age at diagnosis, for most patients, was lower than 12 months of life (85.1%) [17].

Sr. No.	Study	Interviewed Patient	CHD
1	Elmagrpy Z et al., 2011, [15]	1193 (55%)	537 (45.0%)
2	Nisli K et al., 2008, [16]	1042 (59.6%)	421 (40.4%)
3	Vilas Boas LT et al., 2009, [17]	47 (53.2%)	22 (46.8%)
4	Laursen HB, 1976, [18]	1504 (94.7%)	80 (5.3%)
5	Figueroa JDR et al., 2003, [19]	275 (41.8%)	160 (58.2%)
6	Kim MA et al., 2014, [20]	394 (43.1%)	224 (56.9%)

[Table/Fig-5]: Frequency of CHD in Down's syndrome cases found by various researchers.

CHD Cases	Clinically Down's Syndrome Suspect	Down's Syndrome Cases Confirmed
374 (98.13%)	18 (4.81%)	7 (1.87 %) (ECD-3, VSD-2, ASD-1 and PDA-1)

[Table/Fig-6]: Down's syndrome patients in CHD cases investigated in present study.

The other causes of the CHD were not included in the study. The various workers also used other chromosomal abnormality like Turner syndrome, Edward syndrome and Patau syndrome and other. Due to lack of time we were unable to perform karyotyping of the all 374 CHD patients. At primary level we clinically diagnosed 18 Down's syndrome patients, but to overcome this limitation we confirm them by karyotyping this chromosomal analysis substantiate seven patients as Down's syndrome.

CONCLUSION

From this study it can be concluded as Down's syndrome also one of the genetic cause of CHD in the paediatric cases. Down's syndrome can be prevalent in the children with CHD, whereas, the prevalence of Down's syndrome was significantly

very low when evaluated to age-matched control participants. The incidence of Down's syndrome among children with CHD was found to be 1.87%.

ACKNOWLEDGEMENTS

The authors acknowledge the NKP Salve Institute of Medical Sciences and research Centre, Nagpur India, for permitting us and financial support towards chemicals used in research.

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FINANCIAL OR OTHER COMPETING INTERESTS:
As declared above.

Date of Publishing: **Apr 01, 2017**