Original Article

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Cytogenetic Association of Cardiac and Non-Cardiac Anomalies in Down's Syndrome

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Abstract

Introduction: Down syndrome is the most common genetic disorder throughout the world. There is a lot of literature contributed by various studies; however, very few studies have reported the frequency of various types of cytogenetic abnormalities and their association with various congenital defects in the individuals with Down's syndrome

Aim: To establish the clinical and cytogenetic correlation in the Down's syndrome individuals.

Material and Methods: Retrospective analysis was done in 482 individuals with Down syndrome, who were clinically and cytogenetically evaluated during 2003 to 2015 in Genetic and Health Research Centre, Nashik, India.

Results: Out of 482 cases of Down syndrome studied, free trisomy 21 was identified in 422 cases (87.55%), translocation karyotype was observed in 36 cases (7.47%), mosaicism in 22 cases (4.56%) and two cases did not show any obvious chromosomal abnormality. 207 cases showed cardiac anomaly (42.95%), which were Atrio-Ventricular Canal Defect in 105 cases (51%), Ventricular Septal Defect in 62 cases (30%), Atrial Septal Defects and Patent Ductus Arteriosus in 17 cases each (8%) and Tetralogy of Fallot in 6 cases (3%). The frequencies of chromosomal abnormalities observed in Down's syndrome cases with cardiac defects was 89.85% (186/207) for free trisomy 21, 8.17% (18/207) for translocation Down syndrome and 1.45% (3/207) for mosaicism. Non cardiac anomalies were observed in 92 patients (19%) and these were mostly in gastrointestinal tract, including duodenal atresia in 44 cases (9%), imperforate anus in 28 cases (6%) and Hirschsprung's disease in 20 cases (4%). Mosaicism did not show any non-cardiac anomaly.

Conclusion: Among Down syndrome with all types of chromosomal abnormalities, 42.95% cases had cardiac defects and 19.1% had non-cardiac defects. Cases with trisomy exhibit a common association with occurrence of ventricular septal defect while those with mosaicism, which was minimally found, had an association with Atrial Septal defect. Thus, careful clinical evaluation of both cardiac and non-cardiac defects is warranted in all the new-borns with Down syndrome for early intervention and timely managemet.

Keywords: Karyotype; Translocation; Mosaicism.

Introduction

Down syndrome is one of the most common Genetic disorder with the incidence of 1 in 1000 in the world [1]. It is named after John Langdon Down, the British doctor, who fully described the syndrome in 1866 [2]. The clinical cardinal features used to diagnose Down syndrome are the typical facial appearance (flat occiput, flattened facial appearance, brachycephaly, epicanthal folds, flat nasal bridge, upward slanting palpebral fissures, Brushfield spots, small nose and small mouth,

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protruding tongue, small and dysplastic ears), generous nuchal skin, diastasis recti and single transverse palmer crease, short fifth finger with clinodactyly, and a wide space between the first and second toes. Other general features include generalized hypotonia, developmental delay, short stature, learning disability etc [3]. Dr Jerome Lejeune, discovered the genetic cause for Down syndrome in 1958, as having three copies of the genes on chromosome 21, rather than the usual two [4]. The most common cause (about 92-95% of cases) is a complete extra copy of chromosome 21, resulting in trisomy 21 [5]. In 1% to 2.5% of cases, some cells in the body are normal and others have trisomy 21, known as Mosaic Down syndrome [6]. The other common mechanisms that can give rise to Down syndrome include; a Robertsonian translocation, isochromosome or ring chromosome 21 [7]. Robertsonian translocation involving extra chromosome 21 has been shown to be present in 2-4% cases [8]. It is a rare form of chromosomal rearrangement that can occur in the five acrocentric chromosome pairs, namely 13,14,15 (D Group); 21 and 22 (G Group) [9].

People with Down syndrome are at increased risk for certain health problems. Congenital heart defects, increased susceptibility to infection, respiratory and hearing problems, obstructed digestive tract, sleep apnea, Alzheimer's disease and childhood leukemia occur with greater frequency in children with Down syndrome. The rate of congenital heart disease in newborn with Down syndrome is around 40% [10]. Other frequent congenital problems associated with Down's syndrome include Duodenal Atresia, Pyloric Stenosis, Meckel Diverticulum and Imperforate Anus [11]. Constipation occurs in nearly half of people with Down syndrome, one of the potential causes is Hirschsprung's disease, which is due to a lack of nerve cells controlling the colon [12]. Except the Delhi and Banglore studies [13], currently chromosomal studies in Down syndrome in various regions of India are lacking. The current study was therefore undertaken to find out the various types of chromosomal abnormalities in Down syndrome in Maharashtra region and also to correlate the cytogenetic findings with cardiac and non-cardiac defects in these cases.

The aim of our study was to estimate the frequency of different types of chromosomal abnormalities and various associated congenital defects in individuals with clinical features of Down syndrome.

Material and Methods

This is a retrospective study done at Genetic Health and Research Centre, Nashik of the 482 individuals with Down syndrome, from January 2003 to October 2015, which were referred from various parts of the Maharashtra state for confirmation of genetic diagnosis and counseling. The Age of individuals referred ranged from few hours after birth to 15 years.

Only cases with typical physical features of Down syndrome were included in the study. Eight physical features considered for clinical diagnosis included flat face, epicanthic folds, small low placed ears, hypotonia, edema on back of neck, downturned corners of mouth, protruding tongue and increased gap between first and second toes [14]. Children with three to eight above features (Image 1) were included in the study and those with less than three features were excluded from clinical diagnosis of Down syndrome. In the study group, other features of Down syndrome were also recorded subsequently and required investigations were done to check for the associated major congenital cardiac and non-cardiac anomalies.

Image 1: Facial features of a child with Down syndrome

Routine cytogenetic analysis was performed in all the cases by peripheral blood culture and Giemsa banding technique. While analyzing the chromosomes, 20 metaphases were counted and two metaphases were analyzed in free trisomy and translocation cases, whereas 40 metaphases were counted in cases of mosaicism. Chromosomal analysis of parents was also performed in cases of translocation Down's syndrome to check for the possibility of balanced translocations.

Statistical analysis was done by using Z-test for standard error of difference between two proportions (double tailed test) 95% confidence intervals for proportions.

Results

Out of 482 cases studies, chromosomal abnormality was observed in 480 cases and 2 cases did not show any obvious chromosomal abnormality with routine karyotype analysis. Out of the abnormal karyotypes observed, 422 (87.55%) cases had free trisomy 21 (Image 2), in which males were 249 and females were 173. Translocation G/G, D/G karyotypes were observed in 36 (7.47%) cases, affecting 22 males and 14 females and mosaicism was observed in 22 (4.56%) individuals, out of that 10 were males and 12 were females. The overall male to female ratio of Down syndrome cases studied was found to be 1.42 [Table 1].

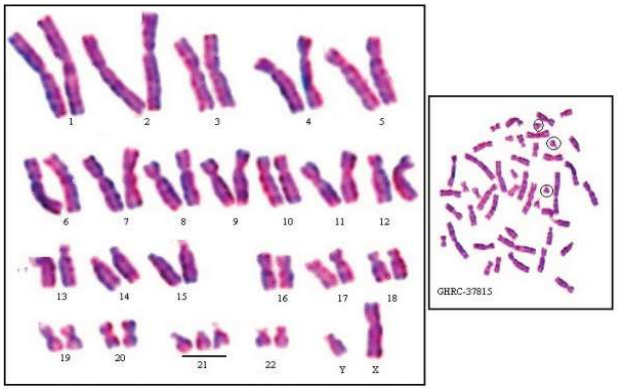

Image 2: Karyotype and metaphase of a patient with free trisomy- 47, XY + 21

Out of 36 translocation cases, 19 showed G/G translocation (52.77%), all of which were homologous translocation of chromosome 21 [t (21;21)] (Image 3). Translocation between 21 and 22 was not seen in any of these cases. 17 (47.22%) cases had a D/G translocation, out of which 13 (36.11%) were between 14 and 21 s[t (14;21)] (Image 4) and 4 (11.11%) were between 21 and 15 [t(15;21)]. Translocation involving 13 and 21 was not seen in any of the individuals.

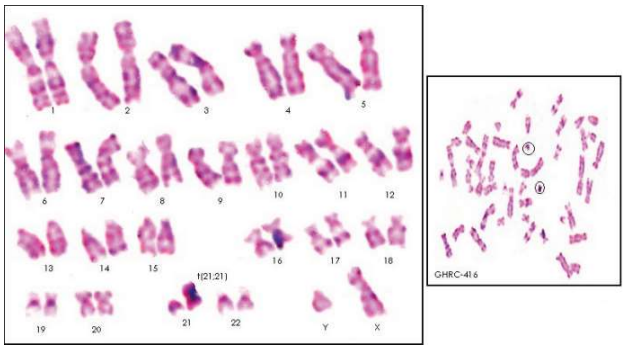

Image 3: Karyotype and metaphase of a patient with Robertsonian homologous translocation- 46, XY, t(21;21) (q11; q11) + 21

Chromosomal analysis of parents of children with Robertsonian translocation Down syndrome (n=36) revealed balanced chromosomal translocation only in 6 parents (16.7%). These translocations included three females all with 14;21 translocation (Images 4a; 4b), three males with 21; 21, 14; 21 and 15; 21 respectively.

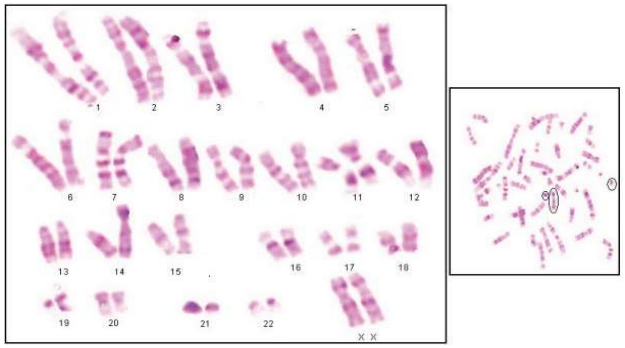

Image 4(a): Karyotype and metaphase of a patient with Robertsonian Translocation (D:G)- 46, XX, t(14;21) (q11;q11) +21

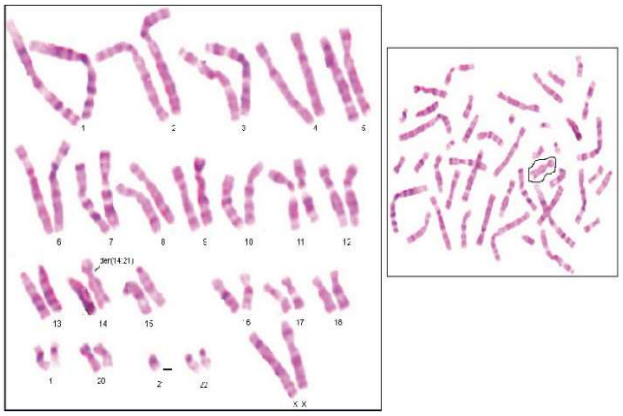

Image 4(b): Karyotype and metaphase of a mother carrier of Robertsonian Translocation (D:G)- 45, XX, t(14;21) (q11;q11)

Clinical evaluation suggests that out of 482 cases, 207 individuals had cardiac anomaly (42.95%) and 92 (19%) had non-cardiac anomaly. Out of 207 individuals, 186 (89.85%) had free trisomy, 18 (8.69%) had Robertsonian translocation and 3 (1.45%) had mosaicism. Other frequent congenital problems included mainly digestive tract anomalies. None of the individuals with Mosaic Down's syndrome had a major non-cardiac anomaly.

The total individuals having cardiac anomalies were 207 (42.95%). The most common cardiac anomaly found was Atrio-Ventricular Septal Defect, observed in 51% (105/207); second most common was Ventricular Septal Defect in 30% cases (62/207), Atrial Septal Defects and Patent Ductus Arteriosus in 8% cases (17/207) each and Tetralogy of Fallot in 3% cases (6/207) [Table 2].

Table 1: Clinical Correlation with the different types of chromosomal abnormalities in Down's syndrome (n=482)

	Types of CA	M	F	Total	Card A	NCar d A	CA in Parents (n=36)
	Free Trisomy 21	249	173	422	186	85	Not available
RT (n=36)	21;21	12	07	19	09	4	45, XY, t(21;21)-1
	21;22	--	--	--	--	--	--
	14;21	07	06	13	07	3	45, XY, t(14;21)-3
							45,XY, t(14;21)-1
	13;21	--	--	--	--	--	
	15;21	03	01	04	02	00	45, XY, t(15;21)-1
Mosaicis m		10	12	22	03	00	Not available
No obvious CA		02	0	02	00	00	Not available
Total		283	199	482	207	92	

CA- Chromosomal abnormality, M- Males, F- Females, Card A- Cardiac Anomalies, NCard A- Noncardiac Anomalies, RT- Robertsonian translocation

Table 2: Various Congenital cardiac Anomalies observed and their association with chromosomal abnormality.

Cardic Anomles(207)	No of Cases	%	Chromosomal Abnormalities		
			Free trisomy	RT	Mosaic
Antriventricular Septal Defect	105	51	95/105 (90.5%)	10 (9.5%)	
Venticular Septal Defect	62	30	60/62 (96.7%)	2 (3.3%)	
Atrial Septal Defects	17	8	13/17 (76.5%)	2 (11.7%)	2 (11.7%)
Patent Ductus Arteriosus	17	8	13/17 (76.5%)	1 (5.9%)	1 (5.9%)
Tetralogy of Fallot	6	3	5/6 (83.3%)	1 (16.7%)	
Total	207	100	186/207 (89.85%)	18	3

RT- Robertsonian translocation

The frequency of chromosomal abnormalities observed in various cardiac anomalies in individuals with Down syndrome for free trisomy is shown in Table 2. The highest frequency of chromosomal abnormality was 96.77% (60/62) observed for Ventricular Septal defect with free trisomy 21 and the lowest frequency of chromosomal abnormalities was 5.88% (1/17) observed for Patent Ductus Arteriosus for mosaicism. Non cardiac anomalies were observed in 92 cases and it was mostly in Gastrointestinal Tract (Table 3.) These include Duodenal Atresia in 44 cases (9%), Imperforate Anus in 28 cases (6%) and Hirschsprung's Disease in 20 cases (4%) (Table 3).

The frequencies of chromosomal abnormalities in various non-cardiac anomalies observed were 80.43% (74/92) for free trisomy 21 and 19.56% (18/92) for Robertsonian translocation cases. Mosaicism did not show any non-cardiac anomaly. The highest frequency of chromosomal abnormality observed was 48.64% (36/74) in Duodenal atresia for free trisomy 21 and lowest was 14.28% (1/7) observed in Hirschprung's disease for t (14;21). [Table 3].

Table 3: Various Non-cardiac Anomalies Observed (Total Number- 92)

Chromos mal abnormality	Duoden al atresia	Imperfor ate anus	Hirschprung's disease	Total (92 cases)	
Free trisomy 21 (422)	36	21	17	74 (80.43%)	
t(21;21) +21(19)	5	3	2	10	18 (19.56%)
t(14;21) +21(13)	3	3	1	7	
t(15;21) +21(4)	-	1	-	1	
Mosaicism (22)	-	-	-	-	
Total	44	28	20	92	

Discussion

The present study conducted on 482 individuals of Down syndrome, shows that the frequency of free trisomy 21 is (422/482) 87.55%, translocation karyotype is (36/482) 7.47% and mosaicism is (22/482) 4.56% respectively. The largest study so far in India, which included 645 individuals with Down syndrome from Delhi has reported free trisomy 21 in 93% (600/645), translocation Down syndrome in 4.1% (26/645) and Mosaicism in 2.6% 917/645) [13]. Another large study from Bangalore on 275 individuals with Down syndrome has reported free trisomy in 87.64%, translocation in 6.55% and Mosaicism in 5.82% of the cases [15]. Our study findings are similar to the studies discussed 2 out of 482 cases did not show any obvious chromosomal abnormality in our study. This might be due to two possible reasons; one could be the undetected mosaicism and another could be the duplication of genetic material on q arm of chromosome 21 [16, 17].

The cytogenetic study conducted on 305 Down syndrome children among Albanian population of Kosovo revealed that free trisomy 21 is significantly more frequent (93.4%) than the other types of trisomy 21 and translocation karyotype was found in 17 cases (5.6%) [16]. Our results are comparable to the results of other studies [19-22].

The proportions of free trisomy and translocation karyotype in this study are significantly different as compared to the largest study in India [13] carried out in Delhi (Z=3.01, p=0.003** & Z=2.36, p=0.018*). However proportion of mosaicism is not much different (Z=1.68, p=0.093). Findings of this study highly match with Bangalore based study [15] for free trisomy (Z=0.04, p=0.968), translocation karyotype (Z=0.48, p=0.6312) and mosaicism (Z=0.74, p=0.4592). The study of Albanian population of Kosovo [16] also shows different proportion of trisomy 21 as compared to this study. (Z=2.83, p=0.005**).

Jayalaksamma et al. reported high presence of Robertsonian translocation between 14 and 21 in children with translocation Down's syndrome (62.34%) [23]. The most frequent type of translocation in our study seen is Robertsonian translocation between 21 and 21 (52.77%); the second most common translocation is between 14 and 21 (36.11%) and then between 15 and 21 (11.11%). S kolgeci in his study on 305 children with Down syndrome also observed Robertsonian translocation 21 and 21 in highest frequency of 58.8%, followed by 14 and 21 in 23.55%, 15 and 21 in 5.9% and 13 and 21 in 5.9% [18].

The incidence of balanced chromosomal translocations in parents of children with Robertsonian translocation in our study is found to be 16.7% (6/36) with equal contribution from maternal and paternal sides. A similar study by Jayalaksamma et al revealed the balanced translocation frequency of 7.8% (12/77 cases) in parents with higher frequency for maternal origin of translocation [23]. The incidence of balanced chromosomal translocation in parents of children with Robertsonian translocation in this study is similar to the study Jayalaksamma et. al. and the comparative values of significance between these two studies are (Z=0.15, p=0.8808).

Out of 18 cases of Robertsonian translocation Down syndrome, Atrial septal defect was seen in 10 cases with the highest frequency of 55.55%, followed by PDA in three cases with a frequency of 16.66%. We could not find any literature reporting the correlation between specific cardiac defect and the translocation type Down syndrome.

Our study indicates that males (283) are affected more than the females (199) with a male to female sex ratio of 1.4:1, which is consistent with previous studies [15,24]. Natalia et al. [25] have found a sex ratio of 1.23 and stated that sex ratio is expected to be higher because of contribution of paternal non-disjunction. Sharav T et al. [26] have reported a high sex ratio in Down syndrome in Jewish population and have stated the possible association of strict practice of long duration of sexual abstinence. We did not find any specific cause for the higher sex ratio in our study. In this study, male to female ratio (Table 1) is 1.42 (95% C.I.1.19 to 1.71) which overlap with the ratio of male to female in the study by Natalia et al. [25] (p>0.05).

Major cardiac anomalies have been reported in more than 40% babies with Down syndrome and the most common one reported is atrio-ventricular septal defect [27-31]. Our study shows cardiac anomalies in 42.95% of individuals with Down syndrome, which is similar to the studies discussed. Freeman et al. has reported atrioventricular septal defect in 45% of individuals with Down syndrome [1], which in our study is observed in 51% of cases. Other problems that may occur include Tetralogy of Fallot and Patent Ductus Arteriosus [11]. We found atrial septal defects and Patent Ductus Arteriosus in 8% individuals and Tetralogy of Fallot in 3%.

The frequencies of chromosomal abnormalities in various cardiac defects observed in our studies were 89.85% for free trisomy 21, 8.70% for Robertsonain translocations and 1.45% for mosaicism. The highest frequency observed was 96.77% for free trisomy 21 in ventricular septal defect and the lowest was 5.88% for mosaicism in Atrial septal defect. There is a study [32] showing the frequencies of various chromosomal abnormalities in the new-borns with specific cardiac defects in syndromic and non-syndromic presentations. However, we did not find any study in the literature showing the frequencies of chromosomal abnormalities in specific cardiac anomalies only in Down syndrome.

Other major congenital anomalies, particularly digestive system anomalies, were also more frequent than in babies without Down syndrome. Duodenal atresia was observed in 5-7% of infants with Down syndrome, as compared to 1 in 1000 infants without Down syndrome [33]. Approximately 3% of infants with Down syndrome are born with an imperforate anus [26,27,30,31]. Hirschprung's disease is a relatively rare condition that is seen in 2.62% of infants with Down's syndrome [34]. In our study we found out duodenal atresia in 9%, imperforate anus in 6% and Hirschprung's disease in 4% of individuals with Down syndrome, these finding roughly are similar to studies discussed (Table 3).

The frequencies of chromosomal abnormalities in various non-cardiac anomalies observed in our study were 80.43% for free trisomy 21 and 19.56% for Robertsonian translocation. Highest frequency observed was 39.13% (36/92) for free trisomy 21 in Duodenal atresia and lowest was 1.09% (1/92) for both t (14; 21) +21 and t (15;21) +21. There are studies reporting the frequencies of various non-cardiac anomalies in Down syndrome [26,27,29-32], however, we could not find any study reporting the frequencies of chromosomal abnormalities in specific non-cardiac defects in Down syndrome individuals.

Future Prospect of the Study

A large multicentric study is required to assess the trend of Down syndrome in various parts of India for assessing the regional distribution, frequencies of individual chromosomal abnormalities in various cardiac and non-cardiac anomalies and also to find out the other genetic and non-genetic factors influencing the occurrence of congenital malformations.

Conclusion

This study reveals that there is a close association between the various types of chromosomal abnormalities in Down syndrome and cardiac and non-cardiac congenital malformations. Our study adds three important findings that have not been reported earlier. These are correlation between specific cardiac defects and Robertsonian Down syndrome; frequencies of chromosomal abnormalities in various types of cardiac defects in Down syndrome and frequencies of chromosomal abnormalities in various non-cardiac anomalies in Down syndrome. It can be concluded that the extra genetic material on chromosome 21 plays a vital role in development of major congenital malformations. However, as not all the individuals with Down syndrome show major malformations in body systems, a very systematic analysis of malformations in cases of Down syndrome is required to find out other factors responsible for absence of malformations in such cases.

Limitations of the study

This study does not include the relationship of maternal and paternal age with the occurrence of Down syndrome and associated malformations.

Ethical Clearance for the study

Since this is a retrospective study based on the available records where the analysis was done, there is no question of revealing the identity as well as there is no harm to any subject ethical clearance is not required. Clearance was obtained from the research committee of Genetic Health & Research Centre, Nashik for using the data for research purpose.

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