Research Article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Molecular Cytogenetic Analysis of Congenital Heart Defects Children with Down syndrome

Bheemshetty Patil[1], Rudragouda S Bulagouda[1] and Gurushantappa S Kadakol[*]

Human Genetics Laboratory Dept. of Anatomy, BLDE (DU)
Shri B M Patil Medical College, Hospital & RC Vijayapur, Karnataka, India.
E mail: dr.patilbs@gmail.com, drravisb2012@gmail.com

ABSTRACT

Down syndrome is the most common genetic condition in the world today and India has one of the highest incidences of Down syndrome. Congenital cardiac disease is the greatest cause of death in patients with Down syndrome during the first two years of life, with from two-fifths to two-thirds of those with Down syndrome also having congenital cardiac malformations. The frequency of congenital heart disease (CHD) among children's with down syndrome (DS) was carried out by G banding & Fluorescence in situ hybridization (FISH) technique. 1 ml of peripheral blood samples were collected in Out Patient Department of pediatrics, Cytogenetic &Molecular analysis was performed. Total 15 samples, out of which 10 were Trisomy 21 and FlorescentIn Situ Hybridization (FISH) Positive and 5 normal cases during this study. All patients were diagnosed CHD by 2- dimensional echocardiography. The present study chromosomal non-disjunction was the most common type of Down syndrome. FISH analysis is easy detection of chromosomal imbalances without a need for metaphase preparations, can be applied to the diagnosis of trisomy 21 and extended to other disorders with chromosomal imbalances.

KEY WORDS: Congenital Heart Defects, Down syndrome, FISH Analysis, G-banding Technique

***Corresponding Author**

Gurushantappa S Kadakol

Research Scientist, Human Genetics Laboratory Dept. of Anatomy, BLDE (DU)

Shri B M Patil Medical College, Hospital & RC Vijayapur-586103, Karnataka, India

Email: nandhishkadakol@gmail.com, Mob no: +916360436363

INTRODUCTION

Down syndrome is the most common genetic condition in the world today and India has one of the highest incidences of Down syndrome. In spite of that, awareness about this condition is so low, most people tend to confuse persons with Down syndrome with other intellectual disabilities.

Congenital cardiac disease is the greatest cause of death in patients with Down syndrome during the first two years of life, with from two-fifths to two-thirds of those with Down syndrome also having congenital cardiac malformations[1].It is important to be familiar with the incidence and anatomical characteristics of CHD in DS, as well as the associated complications and causes of morbidity and mortality, in order to apply preventative measures and to improve the patient's quality of life. In addition, because the type of CHD and the timing of repair affect the prognosis, timely treatment of cardiac abnormalities is crucial for optimal survival[2].

A high frequency of congenital heart disease occurs in children with Down syndrome from a population with widely prevalent consanguinity [3,4] .Prevalence is higher in Asian countries, mostly seen in lower educational and socioeconomic groups, that are traditionally religious albeit declining with modernization. Morbidity increases significantly with inbreeding in many diseases studied in many countries[5]. The risk for birth defects in the offspring of first cousin mating has been increased to 5-8% compared to 2- 3% in non-consanguineous marriages[6]. The prevalence of consanguinity reported in India is 36% and uncle-niece and first cousin are the more commonly occurring relationships in Indian population [7]. In spite of the available literature on consanguinity, there seemed a knowledge gap in India, regarding the consanguinity and DS. Since in North Karnataka region of India consanguineous marriages are widely prevalent, an attempt is made to know the association of CHD in DS.

Current techniques such as amniocentesis and chorionic villi sampling (CVS) require lengthy laboratory culture procedures and high costs. This study to establish a rapid prenatal diagnosis of trisomy 21 using real-time quantitative polymerase chain reaction (PCR) and FISH by patient blood samples[8].The relative levels of syndrome with CHD group (DSCHD) and Non CHD group (CG) is analyzed. The differences between these two groups were statistically analyzed significant (*p*-values). The relative levels of Down syndrome with CHD group (DSCHD) and non CHD group (CG) will be compared.

MATERIALS & METHODS

All patients were diagnosed CHD by 2- dimensional echocardiography and analysed by using karyotyping in combination with Q-PCR and conformed by FISH (Fluorescent In Situ Hybridization)

The study has been conducted in the Laboratory of genetics, department of Anatomy and Department of Pediatrics and we have obtained the Institutional ethical clearance certificate from our hospital Karnataka, in collaboration with Karnataka Institute of DNA Research (KIDNAR) Dharwad.

We have received total 15 samples, out of which 10 were Trisomy 21 and Florescent In Situ Hybridization (FISH) Positive and 5 normal cases.

Inclusion criteria

All the participants were taken from children's of age 0-15 years with the same ethnic origin were selected in this study

Exclusion criteria

Other congenital defects and CHD cases as well as patients from south Karnataka and rest of other states of Indian were excluded from the study.

Respiratory disorders cases were advised 2- dimensional echocardiography and diagnosed as CHD, these cases were selected for analysis of Karyotyping and FISH. After analysis positive cases were selected as subjects and rest of them are normal control

informed consent

Informed consent were taken before the procedure (Patients families were informed about the procedure in detail)

Ethics approval

Study was approved by the institutional ethics committee and informed consent was taken from the parents.

METHODOLOGY

Cytogenetic analysis we collected 1 ml peripheral blood samples from Out Patient Pediatric department. Karyotyping was carried out for peripheral lympho-cytes, cultured from peripheral blood and stained with Giemsa stain as per the Standard Operating Protocol. Olympus Trinocular Research Florescent In Situ Hy-bridization (FISH) with Applied Spectral Imaging Kary-otyping System (Manufacturer name: Olympus, Japan; model: CH20i) was also used during the study. 20-40 spreads were analyzed for each case. The slides were analyzed for detection of chromosomal abnormality for

Down syndrome (Trisomy 21). Fluorescence in situ hybridization (FISH) analysis was carried out with commercially available AneuVysionMulticolour DNA Probe Kit (Vysis LSI 21 Spectrum Orange).

RESULTS

We have received total 15 samples, out of which 10 were Trisomy 21 and Florescent In Situ Hybridization (FISH) Positive and 5 normal cases during this study. All patients were diagnosed CHD by 2- dimensional echocardiography

Out of 10 cases we received males were 60% and females were 40%. Out of 5 normal cases (control) males were 60% and females were 40%. **Table no 1** shows the percentage of male and female cases.

Table1: Percentage Distribution of patients according to Gender.

SUBJECTS	CASES		CONTROL	
	Frequency	Percent	Frequency	Percent
MALE	6	60	6	60
FEMALE	4	40	4	40
TOTAL	10	100	10	100

The maximum and minimum age of patient's mother are 24 and 27 yrs respectively in which the frequency of 24 yrs is 10%, 25 yrs is 30%, 26 yrs is 50% and where as 27 yrs is 10% as per the **table no 2**.In control the maximum and minimum age of patient's mother are 25 and 26 yrs in which the frequency of 25 yrs is 60% and that of 26 yrs is 40%.

Table 2: Percentage Distribution of patients according to Age of mothers

AGE	CASES		CONTROL	
	Frequency	Percent	Frequency	Percent
24	1	10		
25	3	30	3	60
26	5	50	2	40
27	1	10		

We have recorded the age of infant's in **table no 3**. The maximum and minimum age of infants from 13 to 3 days in which the frequency of 1-10 days is 80% and 11-20 days is 20%. In control the maximum and minimum age of infants are 65 and 5 days in which the frequency of 1-10 days is 40%, 21-30 days is 40% and >30 days is 20% .

Table 3: Percentage Distribution of patients according to Age of Infants

DAYS	CASES		CONTROL	
	Frequency	Percent	Frequency	Percent
0-10	8	80	2	40
11-20	2	20	0	0
21-30	0	10	2	40
>30	0	10	1	20

Table 4: Descriptive Statistics

	CASES			CONTROL			CASES		CONTROL		TOTAL	
AGE	No.	Min	Max	No.	Min	Max	Mean	SD	Mean	SD	Mean	SD
Age of Mothers (yrs)	10	24	27	5	25	26	25.6	0.8	25.4	0.5	25.5	0.7
Age of infants (days)	10	3	5	5	5	65	6.6	4.2	26	23.7	13.1	16.2

The frequency of children having Down syndrome whose parents had consanguineous marriage is 70% and with nonconsanguineous is 30%. In control the frequency of children having Down syndrome whose parents had consanguineous marriage is 40% and with nonconsanguineous is 60%, as per **table 5.**

Table 5: Percentage Distribution of patients according to marriages

MARRIAGE	CASES		CONTROL	
	Frequency	Percent	Frequency	Percent
consanguineous	7	70	4	40
Nonconsanguineous	3	30	6	60
Total	10	100	5	100

The 2 dimensional echocardiography study showing variation in the pattern of CHD in down syndrome that is Atrial Septal Defect(ASD), Ventricular Septal Defect(VSD), Patent DuctusArteriosus(PDA) and Tetralogy of Fallot. The frequency of ASD is (20%), VSD is (50%), PDA is (20%) and Tetralogy of Fallot is (10%). As per table **6.**

Table 6: Percentage of Pattern of CHD in children with Down syndrome

CHD	NO OF DEFECTIVE CASES	Percentage OF DISORDERS (%)
ASD	2	20%
VSD	5	50%
PDA	2	20%
Tetralogy of Fallot	1	10%
Total	10	100%

Chromosome analysis was carried out for ten suspected CHD with Down syndrome & 5 normal cases. Ten cases were shows simple trisomy, Mosaic trisomy translocation trisomy was not found.

Table 7: Diagnosis of patients

DIAGNOSIS	N	PERCENTAGE
Down Syndrome	10	66.7
Control group	5	33.3
Total	15	100.0

Fluorescence in situ hybridization (FISH) analysis was carried out with commercially available AneuVysionMulticolour DNA Probe Kit (Vysis LSI 21 Spectrum Orange) with cytogenetic location 21q22.13-q22.2.The results of present study does not show any mosaicism.

DISCUSSION

Down syndrome is most frequently observed autosomal chromosome anomaly in newly born babies. The presence of CHD in Down syndrome patients is a well known fact as it is based on previous literature. The frequency of CHD in Down syndrome varies from 35-65% [9].

The frequency of CHD as per gender in present study is 60% male and 40% female.

The incidence of CHD with Down syndrome in age of infant 0-10 days is 80%, 11-20 days is 20%, 21-30 days is 10% and >30 days is 10%.Our study is correlating with Mohd Ashraf et al in infants (1-21 days)[10].

The mother's age having CHD with Down syndrome infant is minimum 24yrs and maximum 27yrs and their frequencies are 10% and 10% respectively. The incidence is highest at the age of 26yrs having frequency 50% when compared to Emma J et al study showing similar with present study[11].

The CHD is common in Down syndrome. The commonest CHD in Down syndrome is

Ventricular Septal Defect (VSD).In our study the incidence of VSD is 50%, ASD is 20%,PDAis 20%,Tetralogy of Fallot is 10%.The study conducted by Emma J et al, the incidence of CHD in Down Syndrome was 45% ,Freeman SB et al is 48%[12].

Down syndrome has a prevalence of one in 500 to one in 1,000 live births, due to disorder of development arising from incomplete embryogenesis as a result of an additional chromosome 21 copy in the karyotype. This extra chromosome is derived from an over-expression of genetic material due to a tripling of the number of genes. This phenomenon produces structural and functional disorders of the all the systems of the body [13].There are three forms of DS namely simple trisomy, Mosaic trisomy and translocation trisomy. Karyotype analysis of our study finds only the simple trisomy, in which a form of faulty division of reproductive cell (generally occurring during 1st or 2nd meiotic division) prior to or at conception that results in an embryo with three copies of chromosome 21.

Absence of mosaicism in blood does not exclude mosaicism in other tissues. Tissue specific mosaicism has been reported in cases of trisomy 18, trisomy 13 and Turner syndrome. The degree of mosaicism varied between the two tissues in some mosaic individuals, but none of the non-mosaic cases (in blood) was found to be mosaic in the alternate tissue investigated[14].

The commonest incidence of above authors study and present study VSD% and PDA% is comparatively normal whereas ASD% is decreased among North Karnataka people when compared with above authors.

CONCLUSIONS

The Down syndrome with CHD is more common in childrens. The commonest CHD in Down syndrome is Ventricular Septal Defect (VSD) as per our study. In that the north Karnataka population the consanguineous marriages are more common which is one of the cause for chromosomal disorders. Hence Down Syndrome with CHD is more in north Karnataka region. Therefore neonatal screening for the risk of CHD in DS patients must and should be done echocardiography. All children born with DS should have a cardiac evaluation at birth. It is concluded from the present study that chromosomal non-disjunction was the most common type of Down syndrome chromosomal abnormality in our region.The adequate support as per the social educational-economical-regional-financial needs and requirements of the family and as per the specific needs of the DS individuals are well appreciated.FISH analysis is easy detection of chromosomal imbalances without a need for metaphase preparations, can be applied to the

diagnosis of trisomy 21 and extended to other disorders with chromosomal imbalances. Compared to other interphase FISH techniques, it avoids spot-scoring difficulties.

ACKNOWLEDGMENTS

Authors are thankful to family members for their participation in this study. We are also thankful to Department of Anatomy, our Hon,ble Vice Chancellor Dr M S Biradar BLDE (DU) Shri B M Patil Medical College Hospital &Research Centre, Registrar, and Principal for their guidance, support for this study.

REFERENCES

1. Vida VL ,Barnoya J, Larrazabal LA, Gaitan G, de Maria Garcia F, Castañeda AR Congenital cardiac disease in children with Down syndrome in Guatemala.Cardiol Young. 2005;15(3):286-90
2. Formigari R, Di Donato RM, Gargiulo G, Di Carlo D, Feltri C, Picchio FM. Ann ThoracBetter surgical prognosis for patients with complete atrioventricularseptal defect and Down's syndrome.Surg. 2004;78(2):666–672.
3. Becker S, Al-Hakees Z, Molins C, Peterson RConsanguinity and congenital heart disease in Saudi Arabia.Am J Med Genet 2001; 99: 8-13.
4. Teebi AS, Feraag TIGenetic disorders among Arab populations, 1st ed. Oxford University Press, 1997, pp: 102-110
5. Khlat M, Khoury M.Inbreeding and diseases: demographic, genetic, and epidemiologic perspectives.Epidemiol Rev. 1991; 13: 28-40
6. Magnus P, Berg K, Bjerkedal T. Association of parental consanguinity with decreased birth weight and increased rate of early death and congenital malformationClin Genet 1985; 28: 342-445.
7. Nath A, Patil C, Naik VAPrevalence of consanguineous marriages in rural community and its effects on pregnancy outcome. Indian Journal of Community Medicine 2004; 29: 41-43
8. Hoffman JI. Philadelphia.Churchill LivingstoneIncidence, mortality and natural history. Paediatric Cardiology. 2002; 111-131
9. Vilas Boas LT, Albernaz EP, Costa RG. Prevalence of congenital heart defects in patients with Down syndrome in the municipality of Pelotas, Brazil. J Pediatr (Rio J). 2009; 85: 403-407.

10. Ashraf Mohd , MallaReyaz , ChowdharyJaved, MallaMohd , Consanguinity and pattern of congenital heart defects in Down syndromein Kashmir, IndiaAmerican Journal of Scientific and Industrial Research 2010,1(3): 573-577

11. Emma J. Glasson, Angela Jacques, Kingsley Wong, Jenny Bourke, and Helen Leonard Improved Survival in Down Syndrome over the Last 60 Years and theImpact of Perinatal Factors in Recent Decades.The Journal of Pediatrics 2016;V(19):214-220

12. Freeman SB, Taft LF, Dooley KJ, Allran K, Sherman SL, Hassold TJ. Population-based study of congenital heart defects in Down syndrome. Am J Med Genet. 1998;80(3):213–217.

13. Silva MFMC, Kleinhaus ACS. Processoscognitivos e plasticidade cerebral na syndrome de Down [Cognitive processes and brain plasticity in Down syndrome]. Rev Bras Educ Espec. 2006;12(1):123-38.

14. Bittles AH, Bower C, Hussain R, Glasson EJ. The four ages of Down syndrome. Eur J Public Health. 2007;17(2):221-5.