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Original Article

Investigation of DEL22 Frequency with Fluorescent In Situ Hybridization Method in Children with Conotruncal Heart Anomaly

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ABSTRACT

Objective: Conotruncal heart defects represent 10%-15% of congenital heart diseases and mostly include tetralogy of Fallot, pulmonary atresia with ventricular septal defect, truncus arteriosus, and interrupted aortic arch.

Methods: This study aimed to investigate the prevalence of 22q11.2 deletion (Del22) with fluorescent in situ hybridization analysis among children followed with conotruncal heart defects. In 104 cases with conotruncal heart defects, the 22q11.2 region was screened for deletion through the fluorescent in situ hybridization analysis using a probe specific to this region.

Results: The fluorescent in situ hybridization analysis performed in patients with conotruncal heart defects showed that Del22 was present in 3 cases in Group I (70 pts) with isolated cardiopathy (4.3%), 2 cases in Group II (29 pts) with cardiopathy + dysmorphism (6.9%), 2 cases in Group III (2 pts) with cardiopathy + immunodeficiency + dysmorphism (100%), and 1 case in Group IV (3 pts) with cardiopathy + immunodeficiency (33.3%) ($P < .05$). Eight (7.5%) of 104 patients with conotruncal heart defects were found to have Del22.

Conclusions: The results obtained from the present study are compatible with the literature. The clinical manifestation of Del22 is extremely variable. With additional abnormalities such as immunodeficiency and dysmorphic features, Del22 positivity was increasing statistically.

Keywords: Conotruncal heart defects, Del22, FISH, 22q11.2

INTRODUCTION

Conotruncal heart defects (CTHD) include various defects, such as tetralogy of Fallot (TOF), tricuspid atresia, double outlet right ventricle, and transposition of the great arteries. These defects represent 5%-10% of congenital heart diseases and are required to be corrected in the neonatal or childhood period since they often cause severe cyanosis. Congenital aortic arch malformations occur in approximately 80% of all patients with Del22, suggesting that Del22 is an important risk factor for aortic anomalies.[1-3]

Del22 is a chromosomal disorder that causes congenital defects. The most common clinical manifestations are cardiac defects, palate disorders, dysmorphic face, growth disorders, and immunosuppression. The prognosis of Del22 is variable. The wide spectrum of the phenotypes of the syndrome has been previously divided into different sections (DiGeorge syndrome, velocardiofacial syndrome, cardiofacial syndrome, etc.); however, they are known to be etiologically similar and called 22q11.2 deletion or Del22 syndrome.[1-5]

METHODS

Patient Group

A total of 104 cases, who were followed in our hospital's Pediatric Cardiology Outpatient Clinic for CTHD, were divided into 4 groups according to the presence of dysmorphic features and immune system disorders.

Patients with CTHD were included in the study. Metaphase chromosomes obtained from the peripheral blood of the patients were investigated by the fluorescent in situ hybridization (FISH) method using the Del22 probe. This study was approved by the Ethics Committee of the Gaziantep University with number 341/2017.

Fluorescent In Situ Hybridization Analysis

For the FISH analysis, standard metaphase preparations of peripheral blood lymphocytes were used. Genomic DNA was extracted from peripheral blood following standard protocols. It is the 2-Mb commonly deleted region called DiGeorge Critical Zone and is seen in 90% of patients.[6-8] The critical region of a minimum

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of 300-480 kb containing several genes, including TUPLE1, TBX1, SLC25A1, and CLTD, was described within the region. The Del22 screening was performed via FISH method using TUPLE1 probe (Kreatech Diagnostics, Amsterdam, Netherlands) and P250-A1 kit (MRC-Holland, Amsterdam, Netherlands) in accordance with the manufacturer's instructions. The P250 kit consists of 30 different probes targeting the 22q11 region and can be used to detect common and atypical types of deletion.

Measurements and Parameters

Patients diagnosed with CTHD by imaging methods and parents of the younger patients were informed about the subject of the study. A 2-cc blood sample was collected to analyze with the FISH method, technical details of which were given earlier.

Evaluation of the Data

Statistical analysis was performed using MedCalc version 19.6.4 software. Demographic characteristics of the participants were expressed as frequency and percentage. The one-tailed chi-square test (χ^2) was used for detecting Del22 prevalence between groups. A *P* value of <.05 was considered statistically significant.

RESULTS

A total of 104 patients, ages ranging from 10 days to 18 years, were included in the study. The mean age of the patients was 6.3 ± 5.1 years (range 10 days-19 years). The diagnosis of CTHD was made by the pediatric cardiologist on the basis of echocardiography, cardiac catheterization, and cardiac computed tomography or cardiac magnetic resonance results (Table 1). All cases included in the study were evaluated for Del22 using FISH method. Of the cases, 39 were female and 65 (62.5%) were male. The cases, whose peripheral blood samples were studied, were divided into 4 groups according to the cardiac findings, dysmorphic features, and immune system disorders accompanying Del22. Group I, cases with congenital cardiopathy alone; group II, cases with cardiopathy + dysmorphic findings; group III, cases with cardiopathy + dysmorphic findings + immunodeficiency; and group, cases with cardiopathy + immunodeficiency. The classification of patients according to the phenotypic findings is presented in Table 2.

As seen in Table 2, the largest group in the study was group I, the isolated cardiopathy group with 70 cases, which was followed by group II, cardiopathy + dysmorphism group with 29 cases, group III, cardiopathy + dysmorphism + immunodeficiency group with 2 cases, and group IV, cardiopathy + immunodeficiency group with 3 cases. The FISH analysis performed showed that Del22 was present in 3 cases in group I, 2 cases in group II and III, 1 case in group IV. The ratio of male patients was higher in all groups.

Main Points

- Conotruncal heart disorders carry risk of increased 22q11.2 deletion (Del22) frequency.
- Increased Del22 frequency is more prominent with increased multisystem involvement such as conotruncal heart defect, dysmorphic face, and immune disorders.
- 22q11.2 deletion problem is not rare; therefore, it should be considered for every single patient.

Table 1. Conotruncal Heart Disease Type and Number of Patients

Conotruncal Heart Diseases	Number of Cases
Tetralogy of Fallot	85
Isolated tetralogy of Fallot	(55)
With right aortic arch	(17)
With pulmonary atresia	(13)
Double outlet right ventricle	6
Tricuspid atresia	3
Isolated perimembranous ventricular septal defect	3
Others*	8

*Taussig Bing anomaly, atrioventricular septal defect, dilated ascending aorta, right aortic arch, patent ductus arterious, ventricular septal defect, major aortopulmonary collateral arteries, hypoplastic left heart syndrome.

The classification of patients undergoing Del22 analysis according to the type of cardiopathy and accompanying findings and the rate of Del22 detection are given in Table 3. In the group consisting of 67.4% cases with cardiopathy alone, the most common cardiopathies were TOF. Del22 positivity with FISH analysis were 4.3%, 6.9%, 100%, and 33.3% in groups ($P = .029$, χ^2).

The characteristics of 8 cases with Del22 are given in Table 4. Four (50%) of these 8 patients with cardiac defects had TOF, 1 (12.5%) had TOF + right aortic arch, 1 (12.5%) had perimembranous ventricular septal defect, 1 (12.5%) had TOF + pulmonary atresia, and 1 (12.5%) had major aortopulmonary collaterals. A total of 75% of patients with Del22 were found to have TOF. Four cases were accompanied by immunodeficiency and 3 were accompanied by dysmorphic findings.

DISCUSSION

Del22 is a chromosomal disorder that causes congenital defects. The most common clinical manifestations are cardiac defects, palate disorders, dysmorphic face, growth disorders, and

Table 2. Classification of the Cases, Whose Del22 Analysis Was Performed, According to the Phenotypic Findings and Detection Rates of Del22

	Total Number (Female/Male)	*Del22 + Number
Group I	70 (67.4%) (28–42)	3 (4.3%)
Group II	29 (27.9%) (10–19)	2 (6.9%)
Group III	2 (1.9%) (0–2)	2 (100%)
Group IV	3 (2.8%) (1–2)	1 (33.3%)
Total	104 (39–65)	8 (7.5%)

Group I, cardiopathy alone; group II, cardiopathy + dysmorphic findings; group III, cardiopathy + dysmorphic findings + immunodeficiency; and group IV, cardiopathy + immunodeficiency.
*$P = .0029$, chi-squared test between groups.

Table 3. Detection of Del22 According to the Type of Cardiopathy and Accompanying Findings in Patients for Whom Del22 Analysis Was Performed

	Group I		Group II		Group III		Group IV	
	n	Del22+	n	Del22+	n	Del22+	n	Del22+
Tetralogy of Fallot	45	1	5		1	2	2	1
Tetralogy of Fallot with right aortic arch	10	1	7					
Tetralogy of Fallot with pulmonary atresia	4		9	1	1			
Double outlet right ventricle	3		3					
Isolated perimembranous ventricle septal defect			2	1			1	
Truncus arteriosus	3							
Others*	5	1	3					
Total	70		29		2		3	

Group I, cardiopathy alone; group II, cardiopathy + dysmorphic findings; group III, congenital cardiopathy + dysmorphic findings + immunodeficiency; and group IV, cardiopathy + immunodeficiency.
*Taussig Bing anomaly, atrioventricular septal defect, dilated ascending aorta, right aortic arch, patent ductus arterious, ventricular septal defect, major aortopulmonary collateral arteries, hypoplastic left heart syndrome.

immunosuppression. The prognosis of Del22 is variable. Palatal abnormalities causing hypernasal speech, feeding, and swallowing difficulties can be seen in more than 75% of the cases. Most patients are admitted to the hospital with mild dysmorphic facial and vertebral defects. Immunodeficiency is observed in the majority of cases due to thymic aplasia or hypoplasia increasing susceptibility to viral infections. Furthermore, these individuals are more likely to develop autoimmune diseases such as idiopathic thrombocytopenic purpura and juvenile idiopathic arthritis. Neonatal hypocalcemia is seen in half of the patients. It often recovers spontaneously but can reappear in a certain period of life or after several conditions such as an infection, surgery, and pregnancy. Other clinical features include gastrointestinal disorders, hearing loss, renal and dental defects, learning disorders, and psychiatric problems. The wide spectrum of the phenotypes of the syndrome has been previously divided into different sections (DiGeorge syndrome, velocardiofacial syndrome, cardiofacial syndrome, Shprintzen syndrome, conotruncal cardiac anomaly, and CATCH 22); however, they are known to be etiologically similar and called 22q11.2 deletion syndrome or shortly Del22 at present.[1-5]

The FISH method is the most frequently used method to screen syndromes with common pathogenesis that occur due to loss of genetic material in the eleventh region of the long arm of chromosome 22.[5] The loss of genetic material due to deletion and haploinsufficiency increases the variety of clinical findings and causes different phenotypes in generations in the same family.[5-6] In 93% of the cases, the deletion emerges "de novo" or may be inherited in autosomal dominant manner in 6%-25% of the patients.[5]

Goldmuntz[7] reported that the patients with Del22 had cardiac anomalies and most of these defects were CTHD or aortic arch anomalies. Among these, TOF and interruptic aortic arch are the most frequent ones, similar to our study. The prevalence of Del22 in children with CTHD was reported to be 30%.[2] On this basis, Del22 examination is recommended in the routine clinical practice in all rare conotruncal anomalies such as interruptic aortic arch and truncus arteriosus. The age of diagnosis of the syndrome varies from center to center.[2,7]

Alikaşifoğlu et al[8] conducted a study involving 32 patients with clinical features of TOF, truncus arteriosus, outlet ventricular septal defect, transposition, pulmonary atresia, vascular anomalies, and facial dysmorphism and reported the rate of deletion as 6.3%. In a study by Giray et al.,[9] involving 36 patients with congenital heart defects, the rate of patients with Del22 was found

Table 4. Findings of Eight Cases with Del22

	Cardiopathy	Dysmorphic Findings	Immunodeficiency
1	Tetralogy of Fallot	Hypertelorism	Yes
2	Tetralogy of Fallot	Hypertelorism, low-set ears, small mouth	Yes
3	Tetralogy of Fallot	No	Yes
4	Tetralogy of Fallot	No	No
5	Tetralogy of Fallot with right aortic arch	No	No
6	Tetralogy of Fallot with pulmonary atresia	Hypertelorism, small mouth	No
7	Perimembranous ventricle septal defect	No	Yes
8	Major aortopulmonary collaterals	No	No

to be 19.4% with the FISH method. This rate constitutes 14.2% of patients with isolated CTHD and 30.4% of all patients with CTHD.[9]

In a study by Halder et al.[10] in which 146 patients with congenital heart defects requiring surgical treatment were examined with FISH method for Del22 frequency, typical clinical features of Del22 were detected in 87 cases and 59 cases were observed to have isolated heart defects. The authors further detected hemizygous Del22 in 9 (6.16%) of 146 patients whereas no Del22 was found in patients with isolated heart malformation.

Beauchesne et al[11] investigated the frequency of DiGeorge syndrome in 103 patients by FISH analysis and detected TOF in 77 patients, pulmonary atresia in 23 patients, and truncus arteriosus in 3 patients. Six patients (5.8%) were found to have a Del22, similar to our results.

The FISH technique has many advantages over classical cytogenetic techniques in detecting Del22. It is more precise than traditional karyotyping or using high-resolution banding techniques and requires less effort than karyotyping, DNA dosage analysis, or restriction fragment length polymorphism. The FISH technique has been further reported to be useful in prenatal diagnosis using amniotic fluid cells.[12]

The frequency of cardiac malformations in patients with DiGeorge syndrome ranges from 49% to 83%. The CTHDs are the most commonly seen heart diseases, suggesting that the incidence of TOF in these patients may be high. In the study by Fomin et al.[13] the rate of TOF was found to be 50%. In the present study, 83 of the patients with CTHD had TOF. Del22 should be investigated in all patients with heart malformations such as TOF, interruptic aortic arch, septal defects, and truncus arteriosus.

In our study, the results were found to be similar to the literature. An important point in our study is that with added abnormalities such as immunodeficiency and dysmorphic features (e.g., group III), Del22 positivity was increasing. But in every groups had different rate Del22 patients. Such as some cardiac malformations for example the right-sided aortic arch, are not prominent cardiac anomalies and have no pronounced clinical symptoms. Therefore, diagnosis can only be made when genetic testing is performed.[14]

Population-based screening is required to determine the true incidence and prevalence of this syndrome. However, it may be highly expensive to screen a large population, and it is unlikely as it will be ethically questionable. Therefore, it would be more acceptable to screen certain risk groups. Tobias et al.[15] suggested several guidelines to facilitate the early detection of Del22. Several criteria have been recommended by the International Primary Immunodeficiency Diseases Classification Committee to help diagnose DiGeorge syndrome. These criteria mainly refer to immune defects and include the most common clinical features such as immunodeficiency, hypoparathyroidism, CTHD, facial abnormalities, and 22q11.2.[16] Increased awareness of Del22 syndrome, diagnostic guidelines, and a long follow-up period are the most important factors in diagnosing the disease.

CONCLUSION

In conclusion, the clinical presentation of Del22 is highly variable. It is obvious that the probability of being able to diagnose the disease increases if the test is administered to patients with at least one other symptom of the syndrome in addition to the conotruncal anomaly. However, this can prevent the diagnosis of some isolated cases.

Clinical follow-up of the patients positive for Del22 must be carried out through multidisciplinary teamwork.[7,15] Risks such as hypocalcemia, immunity, vascular anomalies must be taken into consideration before surgical operations. Necessary changes in diet and vaccinations must be taken into consideration. Treatment of infections should not be delayed since some patients are more likely to develop recurrent infections due to immunodeficiency. Furthermore, motor, behavior, and speech developmental processes must be followed closely. Improved life quality for the patients can be only possible with the success of well-organized teamwork.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Gaziantep University (Decision No: 341/2017).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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