Prevalence of immunological aberrations and 22q11.2 deletion in children with conotruncal anomalies: A cross-sectional study

Souvik Das[1], Arun Kumar Baranwal[2], Amit Rawat[3], Ashwini Nair[3], Sanjeev Hanumantacharya Naganur[4], Anupriya Kaur[3], Anand Kumar Mishra[5], Ankur Jindal[3], Anit Kaur[3]

[1]Department of Pediatrics, All India Institute of Medical Sciences, Bhubaneshwar, Odisha, India, [2]Department of Pediatric Cardiac Critical Care, Sri Sathya Sai Sanjeevani Hospital, Nava Raipur, Chhattisgarh, India, [3]Department of Pediatrics, Postgraduate Institute of Medical Education and Research, Chandigarh, India, [4]Department of Cardiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India, [5]Department of Cardiothoracic and Vascular Surgery, Postgraduate Institute of Medical Education and Research, Chandigarh, India

ABSTRACT

Introduction : **22q11.2 deletion is associated with conotruncal anomalies and immunological aberrations. Given the common embryonic origin of conotruncus and thymus, conotruncal anomalies may be associated with immunological aberrations irrespective of 22q11.2 deletion. We planned to study the prevalence of immunological aberrations and 22q11.2 deletion among patients with conotruncal anomaly to understand the impact of their interplay.**

Patients and Methods : **Preoperative children (age <12 years) with conotruncal anomalies were evaluated for clinical dysmorphism, lymphocyte subsets by flowcytometry, immunoglobulin levels by nephelometry, and 22q11.2 deletion by multiplex ligand-dependent probe amplification (January 2021–June 2022). Patients with asplenia and polysplenia were excluded from immunological studies.**

Results : **Major cardiac defects ([*n* = 101], [median age, 32 days]) included dextro-transposition of great arteries (d-TGA) - 41.6%, tetralogy of Fallot - 37.6%, double outlet right ventricle (DORV) - 13.9%, and truncus arteriosus - 4.9%. Four patients had polysplenia with situs inversus, while 17 had clinical dysmorphism. Flow cytometry (*n* = 82) revealed low absolute counts of lymphocytes (33%), T-cells (51.2%), CD4+ cells (50%), and CD8+ cells (51.2%), while only 14.1% had low IgG levels. Eight patients (8/95, 8.4%) had 22q11.2 deletion, with universal deletion of *TBX1-2* and *TBX1-7 genes*; the other 19 genes were deleted in various combinations. Two patients with 22q11.2 deletion had normal T-cell subsets, while none had a complete absence of T-cells.**

Conclusion : **Immunological aberrations, especially T-cell abnormalities, were present in almost half of the patients, irrespective of 22q11.2 deletion. Only 8.4% of patients had 22q11.2 deletion. The high incidence of d-TGA among 22q11.2 deletion patients needs further exploration.**

Keywords : 22q11.2 deletion, conotruncal anomalies, Di George syndrome, immunological aberrations, lymphocyte counts

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Address for correspondence: Prof. Arun Kumar Baranwal, Department of Pediatric Cardiac Critical Care, Sri Sathya Sai Sanjeevani Hospital, Nava Raipur, Chhattisgarh, India.

E-mail: baranwal1970@gmail.com

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INTRODUCTION

Clinical features of 22q11.2 deletion syndrome result from impaired embryonic development of structures originating from the 3rd and 4th pharyngeal pouches. Conotruncal anomalies and immunological aberrations commonly coexist in these patients, as 3rd and 4th pharyngeal arch arteries develop into cardiac outflow tracts while the 3rd pharyngeal pouch gives rise to the thymus. 22q11.2 deletion is associated with a full T-cell spectrum, ranging from normal numbers and function to severe combined immunodeficiency-like presentation.[1-3] Similarly, children with conotruncal anomalies frequently have low T-cell subsets, especially T-helper cells, immunoglobulin (immunoglobulin G, immunoglobulin A), and complement (complement 3 and complement 4) levels, and are predisposed to infections.[4] However, conotruncal anomalies are present only in 50% of children with 22q11.2 deletion, while 22q11.2 deletion is present in 5%–48% of children with conotruncal anomalies.[5-13] Furthermore, known deletions were reported to be absent in about 10% of clinically diagnosed 22q11.2 deletion syndrome.[14] These findings question 22q11.2 deletion being the only genotypic explanation for conotruncal anomalies and suggest the involvement of other genetic loci in the embryonic co-development of conotruncus and thymus.[5-12,14] Thus, patients with conotruncal anomalies are likely to have immunological aberrations irrespective of 22q11.2 deletion.[4] In all but one previous study, the immunological profile was studied in relation to the 22q11.2 deletion.[4-12] However, the presence of conotruncal anomalies might clinically be a more relevant starting point.

Data on the interplay between conotruncal anomalies, immunological aberrations, and 22q11.2 deletion and its impact on immediate post-operative and long-term clinical outcomes are scanty. It is imperative to study the potential of preoperative and postoperative infections and postbypass systemic inflammatory response in patients with conotruncal anomalies due to underlying immunological aberrations. Early detection of immunological aberrations may help in preoperative prognostication and modify the overall clinical care to improve immediate postoperative and long-term survival. The current study was planned to study the prevalence of immunological aberrations and 22q11.2 deletion among children with conotruncal anomalies and the relationship between them, if any.

PATIENTS AND METHODS

Study setting, study design, eligibility criteria, and definitions

This cross-sectional study was conducted in a 1950-bed federally-funded multispecialty tertiary care teaching hospital in a lower-middle income country. All preoperative patients (age <12 years) diagnosed with conotruncal anomalies on echocardiography were planned to be enrolled from the pediatric emergency room, the newly-started preoperative pediatric cardiac critical care unit (named as High Dependency Facility), and outpatient clinics of Departments of Cardiology and Cardiothoracic and Vascular Surgery over 18 months (January 2021–June 2022). Conotruncal anomalies were defined as tetralogy of Fallot (TOF), truncus arteriosus, double outlet right ventricle (DORV), double outlet left ventricle, dextro-transposition of great arteries (d-TGA), conoventricular septal defects, and interrupted aortic arch type B. Immunological studies were not performed among patients with asplenia and polysplenia. Patients whose parents did not give consent were excluded.

Consent

After explaining the purpose of the study, written informed consent was obtained from the legal guardians of eligible children.

Data collection and laboratory investigations

Demographic data, anthropometry, dysmorphism, detailed in-hospital echocardiographic findings, past medical history, and presence of other congenital malformations were extracted in a predesigned proforma. Abdominal ultrasonographic examination and spine radiograph were obtained to look for polysplenia, asplenia, and other malformations. Four ml of whole blood was sampled – 2 ml of anti-coagulated blood for peripheral blood lymphocyte subset analysis (CD45, CD3, CD4, CD8, CD19, and CD56/16 markers), and multiplex ligation-dependent probe amplification (MLPA) for 22q11.2 deletion and 2 ml clotted blood for serum immunoglobulin levels (immunoglobulin G, immunoglobulin A, and immunoglobulin M). Laboratory methods employed are detailed in Supplemental Material 1. Absolute counts of lymphocyte subsets were compared with age-specific reference ranges in healthy children.[15] A value <10th centile was considered to be low. Serum levels of immunoglobulin isotypes were compared with age- and gender-based reference levels in healthy children.[16]

Statistical analysis

Based on our experience, a convenient sample size of 100 was planned. Statistical analysis was done with SPSS (Statistical Package for the Social Sciences) version 25 (IBM Crop. 2017; Armonk, New York, USA). Fisher's exact test was used for intergroup comparisons. P <0.05 indicated significance.

RESULTS

A total of 101 children (76 [75.2%] boys, 25 [24.8%] girls) with conotruncal anomalies were enrolled; four

had polysplenia with situs inversus. The median age was 32 days (interquartile range, 10.5, 99); 59 (58.4%) of them were neonates, 23 (22.8%) were between 1 and 6 months, 5 (4.9%) were 6 months–1 year, while 14 (13.8%) were more than 1 year of age. d-TGA was the most common conotruncal anomaly ($n = 42$, 41.6%), followed by TOF ($n = 38$, 37.6%), DORV ($n = 14$, 13.9%), and truncus arteriosus ($n = 5$, 4.9%). Interrupted aortic arch type B and double outlet left ventricle were present in one each. Sixteen patients with d-TGA had intact ventricular septum (16/42, 38.1%). Additional cardiac defects in these patients included atrial septal defects ($n = 44$, 43.6%), patent ductus arteriosus ($n = 46$, 45.5%), pulmonary atresia (13, 12.9%), and pulmonary stenosis ($n = 11$, 10.9%). Dysmorphic features were seen in 17 children (16.9%), of whom 8 (7.8%) had facial dysmorphism (cleft lip and palate, $n = 2$), 3 (3%) had abnormal hands and feet, and one each had cryptorchidism and hypospadias. Microcephaly (head circumference, <–3 *Z*-score) was present in 21 (20.8%) patients, while another 14 (13.8%) had small heads (head circumference, <–2 to –3 *Z*-score). Four (3.96%) children had renal anomalies (ectopic kidneys, $n = 2$; hydronephrosis, $n = 2$). Spine radiograph did not reveal vertebral anomaly in any of the patients. None of the parents were noted to have dysmorphic features or a history of congenital heart disease.

Immunological studies were not performed for four patients with polysplenia, while technical errors were encountered in 15 blood samples. Thus, data on lymphocyte subsets are available for only 82 patients. Assessment of immunoglobulin isotopes could be done in 71 patients only, as samples were either insufficient ($n = 12$) or got hemolyzed ($n = 14$). Further, levels of immunoglobulin A and immunoglobulin M were below the minimum detectable value in 51 patients for whom samples were processed after dilution. Hence, we only present immunoglobulin A and immunoglobulin M levels of 20 patients [Figure 1].

Data on lymphocyte subsets and immunoglobulin isotopes with a comparison between 22q11.2 deletion-positive patients and 22q11.2 deletion-negative patients are shown in Table 1. Absolute lymphocyte count was low in 35.3% of patients, with similar distribution among patients with d-TGA, TOF, and DORV. Absolute T-cell (CD3+) counts and absolute $CD4^+$ and $CD8^+$ T-cell counts were low in half of the patients, with almost similar distribution in patients with d-TGA, TOF, and DORV. However, the counts of all these cells were low in proportionately more children with truncus arteriosus ($CD3^+$ cells, 75%; $CD4^+$ cells, 100%; and $CD8^+$ cells, 75%). Absolute natural killer cell count was low in about a third of the patients. Absolute B-cell count and immunoglobulin G levels were low in 25.6% and 14.4% of patients. Although denominators were different, immunoglobulin A level was low in proportionately more children compared to immunoglobulin G and immunoglobulin M. Proportionately more children with truncus arteriosus ($n = 4$) had low absolute natural killer cell count (50% vs. 26.5% in d-TGA [$n = 34$] and 36.4% in DORV [$n = 11$]) and immunoglobulin G levels (33% vs. <20%). Two children with 22q11.2 deletion had normal T-cell subsets, while none had a complete absence of T cells. Due to a small number of 22q11.2 deletion-positive patients, differences observed between patients with 22q11.2 deletion and those without could not reach statistical significance [Table 1].

MLPA could be performed on 95 patients only. Samples of six patients got hemolyzed, and these patients were not available for repeat sampling. Eight patients (8.4%) had 22q11.2 deletion with universal deletion of *TBX1-2* and *TBX1-7 genes*. Four patients had a deletion of the same 14 *genes*, while another had an additional gene (*HIC2*-2) deleted. Two patients had a deletion of three genes only. The former five patients (four patients with deletion of 14 genes and one with additional deletion of *HIC2*-2 gene) (5/8, 62.5%) had TOF while the remaining 3 patients (3/8, 37.5%) had d-TGA [Supplemental Material 2].

Four out of five TOF patients had broad nasal bridges, while three had microcephaly. The most common location involved in the deletion was 22q11.21 (88/92; 96.7%). Among 22q11.2 deletion-positive patients, absolute lymphocyte count was low in 37.5% of patients, while $CD3^+$ cell and $CD4^+$ cell counts were low in 75% of patients, and CD8 cell count was low in 37.5% of patients [Table 2].

DISCUSSION

More than half of patients with conotruncal anomalies had immunological aberrations irrespective of underlying 22q11.2 deletion. The 22q11.2 deletion was present in 8.4% (8/95) patients only. The presence of 22q11.2 deletion is found to have a higher incidence of low counts of T-cells and $CD4^+$ T-cells. Studied patients with truncus arteriosus had low values for almost all immunological parameters. In a nutshell, children with conotruncal anomalies seem to cluster around lower immunocompetence parameters tested and may be predisposed to preoperative and postoperative infections.

In a similar study, the percentage of T-cells and CD4+ T-cells were low in children with conotruncal anomalies ($n = 18$) irrespective of the presence of dysmorphic features, while these were normal among patients with shunt lesions ($n = 22$).[4] Immunological aberrations in 22q11.2 deletion-positive patients vary from more common partial defects (normal or

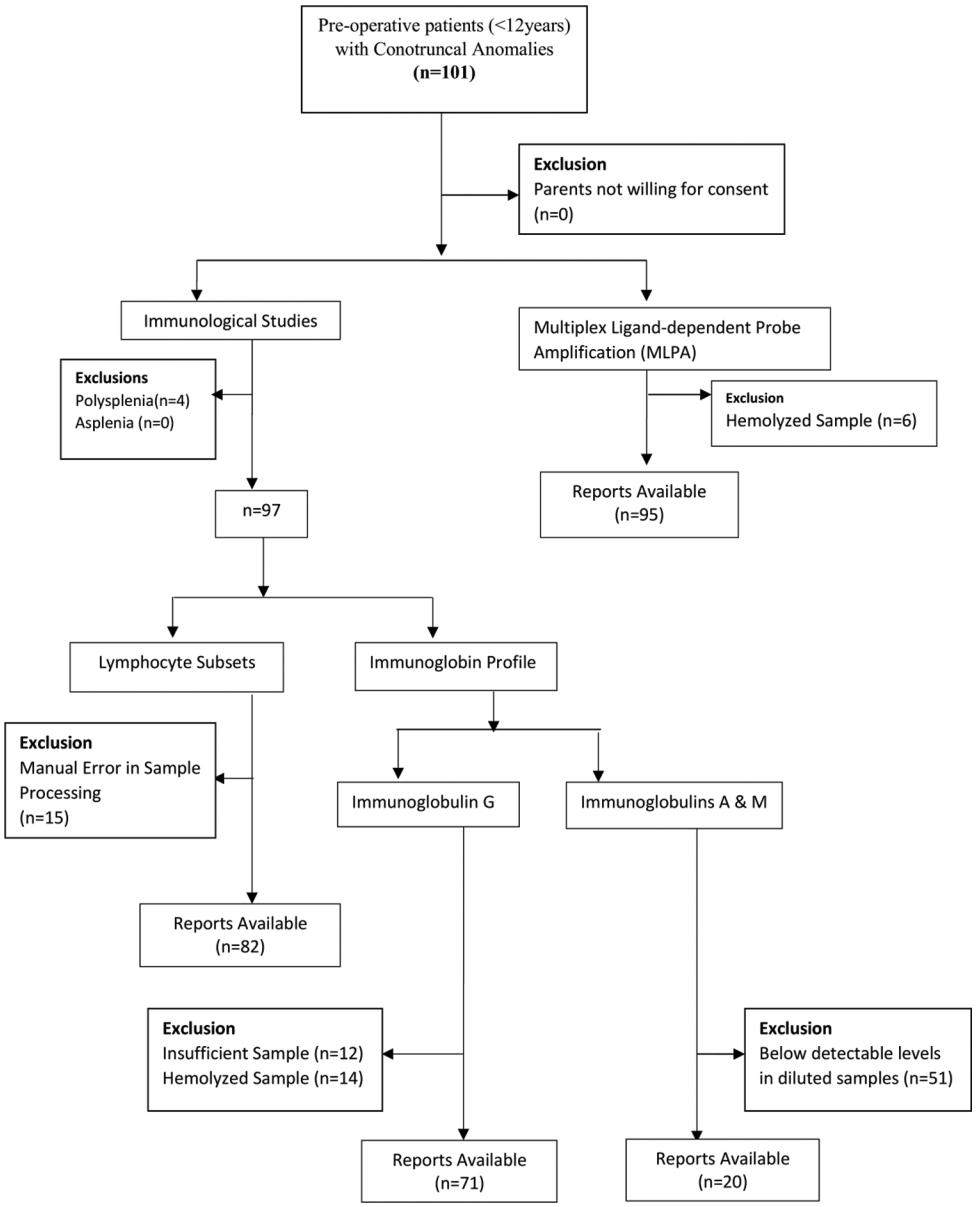

Figure 1: Study flow diagram

attenuated T-cell number and function) to the complete absence of T-cells.[1,3,17] However, none of the 22q11.2 deletion-positive patients in the index study had a complete absence of T-cells. Dysregulated immune response due to immunological aberrations, more so among 22q11.2 deletion-positive patients, are likely to predispose to perioperative infections and other complications due to exaggerated systemic inflammatory response.[18] The number of children with conotruncal anomalies requiring hospitalization for preoperative infections was twice the number of patients with shunt lesions.[4] Similar findings were reported in other studies as well.[19,20] The 22q11.2 deletion-positive patients have a higher incidence of postoperative complications, longer intensive care unit and hospital stays, and higher mortality.[18,21] Patients with low absolute T-cell, CD4+, and CD8+ cell counts are likely to have higher incidence and severity of postoperative systemic inflammation and sepsis.[22] Patients were also found to have recurrent pneumonia and sepsis even after discharge.[8,18,22] Even on long-term follow-up, they are reported to have recurrent acute respiratory tract infections, restrictive lung disease, poor aerobic capacity, higher medication use, and more hospitalizations.[10] Low counts of naïve T-helper cells, naïve T-regulator cells, naïve cytotoxic T-cells, and persistence of low T-cell receptor excision circles in the grown-ups may explain these observations.[23]

Such postoperative trends assume more importance in low-and middle-income countries where children have significant preoperative morbidities, e.g., acute-on-chronic heart failure, recurrent acute

Table 1: Lymphocyte subset and immunoglobulin profile among patients with conotruncal anomalies in relation to 22q11.2 *deletion* positivity (*n*=82)*

	All patients (*n*=82)*				d-TGA (*n*=34)				TOF (*n*=33)				DORV (*n*=11)	TA (*n*=4)
	Total (*n*=82)*, *n* (%)	22q11.2*del*– (*n*=74), *n* (%)	22q11.2*del*+ (*n*=8), *n* (%)	*P*	Total (*n*=34), *n* (%)	22q11.2*del*– (*n*=31), *n* (%)	22q11.2*del*+ (*n*=3), *n* (%)	*P*	Total (*n*=33), *n* (%)	22q11.2*del*– (*n*=28), *n* (%)	22q11.2*del*+ (*n*=5), *n* (%)	*P*	22q11.2*del*– (*n*=11), *n* (%)	22q11.2*del*– (*n*=4), *n* (%)
Low total lymphocytes (CD45+)	29 (35.3)	26 (35.1)	3 (37.5)	1.00	12 (35.3)	10 (32.2)	2 (66.6)	0.28	10 (30.3)	9 (32.1)	1 (20)	1.00	4 (36.4)	3 (75)
Low CD3+ T cells	42 (51.2)	36 (48.6)	6 (75)	0.27	17 (50)	15 (48.3)	2 (66.6)	1.00	16 (51.5)	12 (42.8)	4 (80)	0.18	6 (54.5)	3 (75)
Low CD4+ T cells	41 (50)	35 (47.3)	6 (75)	0.26	16 (47.1)	14 (45.1)	2 (66.6)	0.59	16 (48.5)	12 (42.8)	4 (80)	0.17	5 (45.5)	4 (100)
Low CD8+ T cells	42 (51.2)	39 (52.7)	3 (37.5)	0.48	16 (47.1)	14 (45.1)	2 (66.6)	0.59	15 (45.5)	14 (50)	1 (20)	0.35	8 (72.7)	3 (75)
Low B cells (CD19+)	21 (25.6)	21 (28.3)	0	0.11	10 (29.4)	10 (32.2)	0	0.54	8 (24.2)	8 (28.5)	0	0.30	2 (18.2)	1 (25)
Low NK cells (CD56/16+)	24 (29.3)	22 (29.7)	2 (25)	1.00	9 (26.5)	8 (25.8)	1 (33.3)	1.00	9 (23.7)	9 (32.1)	0	0.29	4 (36.4)	2 (50)
IgG	*n*=71	*n*=63	*n*=8		*n*=28	*n*=25	*n*=3		*n*=31	*n*=26	*n*=5		*n*=9	*n*=3
Low IgG	10 (14.1)	9 (14.2)	1 (12.5)	1.00	5 (17.9)	4 (16)	1 (33.3)	0.46	3 (9.7)	3 (11.5)	0	1.00	1 (11.1)	1 (33.3)
IgA and IgM	*n*=20	*n*=16	*n*=4		*n*=8	*n*=8	*n*=0		*n*=9	*n*=5	*n*=4		*n*=3	*n*=0
Low IgA	7 (35)	7 (43.7)	0	0.25	3 (37.5)	3 (37.5)	-	-	3 (33.3)	3 (60)	0	0.17	1 (33.3)	-
Low IgM	4 (20)	4 (25)	0	0.54	2 (25)	2 (25)	-	-	2 (22.2)	2 (40)	0	0.44	0	-

*Lymphocyte subsets could be done in 82 patients only. d-TGA: Dextro-Transposition of Great Arteries, TOF: Tetralogy of Fallot, DORV: Double outlet right ventricle, TA: Truncus arteriosus, IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M, NK: Natural killer

Table 2: Clinical and immunological profile of patients with 22q11.2 *deletion* (*n*=8)

Patient number	Age/sex	Cardiac disease	Dysmorphism	Total lymphocyte count (CD45+)	CD3+ count	CD4+ count	CD8+ count	CD19+ count	CD56/16+ count	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	Serum calcium (mg/dL)
95	11 months/ male	TOF	Broad nasal bridge	4060 (normal)	1600 (↓)	903 (↓)	575 (normal)	771 (normal)	296 (normal)	769.5 (normal)	NA	84.2 (normal)	7.2 (↓)
6	2 months/ male	TOF, VSD, PS, PDA	Hands and feet, microcephaly	4218 (normal)	3605 (normal)	1982 (normal)	984 (normal)	343 (normal)	219 (normal)	2120 (normal)	452 (normal)	140.8 (normal)	6.6 (↓)
82	25 days/ male	TOF, PDA, PS	Broad nasal bridge, microcephaly	4673 (normal)	645 (↓)	338 (↓)	327 (↓)	2233 (normal)	1192 (normal)	1010 (normal)	213 (normal)	132 (normal)	7.2 (↓)
81	5 months/ male	TOF, PA, aberrant origin of pulmonary arteries	Broad nasal bridge, microcephaly, bilateral Grade II hydronephrosis and Grade V vesicoureteric reflux	3139 (↓)	1632 (↓)	693 (↓)	731 (normal)	922 (normal)	304 (normal)	499 (normal)	64.3 (normal)	68.3 (normal)	9.3 (normal)
58	4 years/ male	TOF, VSD, PA	Broad nasal bridge	2092 (normal)	1087 (↓)	464 (↓)	483 (normal)	616 (normal)	204 (normal)	622 (normal)	187.5 (normal)	277.5 (normal)	7.8 (normal)
43	30 days/ male	d-TGA, ASD, VSD	Low set ears	4012 (normal)	3089 (normal)	269 (normal)	124 (normal)	585 (normal)	100 (↓)	203 (↓)	NA	NA	8.7 (normal)
14	56 days/ male	d-TGA, ASD, VSD	Long philtrum, telecanthus, microcephaly	1717 (↓)	982 (↓)	637 (↓)	195 (↓)	348 (normal)	290 (normal)	1194 (normal)	NA	146.5 (normal)	8.6 (normal)
40	3 days/ male	d-TGA, ASD, VSD, PDA	None	2969 (↓)	1977 (↓)	1294 (↓)	483 (↓)	362 (normal)	564 (normal)	664 (normal)	NA	108.3 (normal)	10 (normal)
			Abnormal values, *n* (%)	3/8 (37.5)	6/8 (75)	6/8 (75)	3/8 (37.7)	0/8 (0)	1/8 (12.5)	1/8 (12.5)	0/4 (0)	0/7 (0)	3/8 (32.5)

TOF: Tetralogy of Fallot, VSD: Ventricular septal defect, PS: Pulmonary stenosis, PDA: Patent ductus arteriosus, PA: Pulmonary atresia, d-TGA: Dextro-Transposition of Great Arteries, ASD: Atrial septal defect, IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M, ↓: Low, NA: Not available

respiratory infections, malnutrition, and anemia due to neglect and late presentation. In these patients, prolonged stay in the intensive care unit may result in poor neurological outcomes, while postdischarge recurrent infections may adversely affect their catch-up growth and development. In a resource-limited setting, it is rational to direct the resources to children likely to have a less stormy postoperative course and better potential for growth and development in subsequent years. However, what constitutes significant immunological aberrations in the context of conotruncal anomalies to indicate a suboptimal postoperative outcome will need long-term follow-up studies with a larger sample size. While early surgery in these patients may be relevant to prevent preoperative infective complications,[8] it may be prudent to take extra precautionary measures to prevent post-operative infections (namely, antibiotic prophylaxis, hygiene, and immunization).

Universal preoperative screening for lymphocyte subsets in patients with conotruncal anomalies may help detect the presence and severity of immunological aberrations. Longitudinal studies will likely reveal the impact of thymectomy (partial or complete) on the preexisting immunological aberrations and the immediate and long-term post-operative outcome. It may also be prudent to compare the severity of postoperative systemic inflammatory response, infections, and associated organ dysfunction between patients with immunological aberrations and those without, after factoring in the presence and extent of surgical thymectomy. Information obtained may be helpful in counseling parents for long-term clinical outcomes and optimal utilization of scarce resources available for the care of children with CHD in low-and middle-income countries. Vaccination against respiratory pathogens (e.g. RSV, *Hemophilus*, and pneumococcus) may be ensured, and patients should be monitored well into adulthood.[24,25]

Reports show the varied prevalence of 22q11.2 deletion based on the type of conotruncal anomalies studied — 7% (conotruncal anomalies with the exclusion of DORV, n = 214),[8] 10% (conotruncal anomalies underwent surgery, n = 177),[9] 18% (TOF, n = 165),[10] and 48% (conotruncal anomalies with exclusion of d-TGA and left outflow tract obstruction, n = 104)[26] In a study from India, 22q11.2 deletion was present in 12.8% of patients with conotruncal anomalies (n = 70); all 22q11.2 deletion-positive patients had TOF and dysmorphism.[27] TOF was also the most common association (5/8, 62.5%) in the index study. Three patients with 22q11.2 deletion (3/8, 37.5%) had d-TGA, which is reported to be rare among children with 22q11.2 deletion from populations of oriental and European descent (up to 1% and 4%, respectively).[1,5,28-31] Although the sample size is small, it may suggest different genetic make-up among Indian children. *TXB1*-2 and *TXB1*-7 were the most common genes deleted, as reported before.[27,32,33] The presence of 22q11.2 deletion only in 8.4% of our patients, while the presence of immunological aberrations in the majority suggests the possibility of other genetic mutations that may directly affect or indirectly modify pharyngeal arch development and thus may present with immunological and phenotypic features consistent with 22q11.2 deletion. Deletions and duplications in other loci or chromosomes (e.g., 22q13.33, 14q32.13, 19p13.3) have been observed in many patients with phenotypic features of DiGeorge Syndrome without 22q11.2 deletion.[34,35] Genes in these locations may also be primarily responsible for the embryonic development of pharyngeal arches or acting as modifiers for the expression of known genes involved in development of pharyngeal arches and/or for 22q11.2 deletion syndrome. Phenotypic expression of *Crkol* deletion is one such example.[13]

The index study represents one of the few studies simultaneously evaluating immunological profile and 22q11.2 deletion status in children with conotruncal anomalies and the first one from the Indian subcontinent. Hemodynamic instability, short preoperative hospital stay, or untimely demise prevented detailed clinical assessment for dysmorphism in many babies. Flow cytometry and immunoglobulin assessment could not be assessed for many patients due to late processing, inadequate quantity, or hemolysis of the blood samples. Ultrasonographic examination of the thymus, Vitamin D serum levels, and parathyroid hormone would have provided alternative markers for 22q11.2 deletion syndrome/DiGeorge Syndrome. Secondary immunodeficiency (e.g. HIV-AIDS) should have been ruled out. Whole exome sequencing in children without 22q11.2 deletion could have detected other responsible genetic abnormalities. Larger studies on the impact of preoperative immunological aberrations on the immediate postoperative outcome, long-term survival and its quality, and immunological status during adolescence and adulthood are suggested to improve clinical decision-making and prognostication.

CONCLUSIONS

This pilot study provides a glimpse into the immunological status of children with conotruncal anomalies. Immunological aberrations, especially T-cell abnormalities, seem to be widely prevalent irrespective of 22q11.2 deletion. The presence of 22q11.2 deletion increases the prevalence of immunological aberrations. The high incidence of d-TGA among 22q11.2 deletion patients needs further exploration. Studies with larger sample sizes and long-term follow-up are required to recommend pre-operative screening for lymphocyte subsets for immediate postoperative and long-term prognostication.

Ethical Standards

The Institute Thesis Committee (Email communication dated 09/01/2021) and the Institute Ethics Committee (letter no. INT/IEC/2021/SPL-242 dated 13/02/2021) approved the study protocol. The Departmental Review Board approved the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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SUPPLEMENTAL MATERIALS

Supplemental Material 1: Laboratory methods

Lymphocyte subset assessment was done with the Stain-Lyse-Wash protocol. Briefly, 100μL of anti-coagulated blood was taken in two FACS tubes and 20 μL of each antibody (CD45, CD3, CD4, CD8, CD19, CD56/16 markers) was added and vortexed well to mix it properly. Tubes were then incubated at room temperature for 20–30 minutes in the dark. After the incubation was over, 1000μL of freshly prepared 1X RBC lysis buffer was added and again vortexed for proper mixing. Tubes were further incubated for 10–15 minutes at room temperature in the dark. After this incubation tubes were centrifuged at 1500rpm for 5 minutes. The supernatant was discarded and 1000μL of Phosphate Buffered Saline was added to tubes followed by centrifugation at 1500rpm for 5 minutes. This step of washing was performed twice. After washing, the pellet was resuspended in 300μL Phosphate-Buffered Saline and acquired on a flowcytometer. Flowcytometry was performed using Navios flowcytometer (Beckman Coulter) and data were analyzed using the Kaluza analysis software (Version 2.2). Results of the flowcytometry data revealed percentages of various lymphocyte subsets which were multiplied with absolute lymphocyte count to get absolute counts of different lymphocyte subsets.

Immunoglobulin isotypes (Immunoglobulin G, Immunoglobulin A, and Immunoglobulin M) were estimated on a fully automated nephelometer (Atellica NEPH 630 system, Siemens Healthineers Ltd.).

Multiplex Ligation-dependent Probe Amplification (MLPA) was done after extracting genomic DNA with QIAmp DNA Blood Mini kit (QIAGEN, Hilden, Germany). Isolated DNA was quantified using Nanoquant (Infinite 200 series, Tecan Trading AG, Switzerland) for checking concentration and quality. Following which, Multiplex Ligation-dependent Probe Amplification was performed with steps involving DNA denaturation (98°C for 5 minutes), hybridization (95°C for 1 minute; 60°C for 16 minutes), ligation (54°C for 15 minutes, 98°C for 5 minutes, bring down temperature to 20°C), Polymerase Chain Reaction amplification (35 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds), and finally the reaction was stopped after bringing the temperature to 72°C. Further, the obtained Multiplex Ligation-dependent Probe Amplification product (0.7μl) was mixed with 8.9μl of highly deionized formamide and 0.4μL of DNA standard LIZ 600 and allowed to denature at 95°C for 2 minutes following which the mixture was loaded on ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The data obtained was then analyzed using Coffalyser.net software.

Supplemental Material 2: Deleted genes in patients with 22q11.2 *deletion* (*n*=8)

Patient number	Number of deleted genes (*n*)	Name of deleted genes	Cardiac defects
95	14	*TBX1-2, TBX1-7, CLTCL1-3, HIRA-25, CDC45-1, CLDN5-1, GP1BB-2, TXNRD2-9, DGCR8-2, ZNF74-2, KLHL22-2, MED15-10, SNAP29-5, LZTR1-16*	TOF
6	14	*TBX1-2, TBX1-7, CLTCL1-3, HIRA-25, CDC45-1, CLDN5-1, GP1BB-2, TXNRD2-9, DGCR8-2, ZNF74-2, KLHL22-2, MED15-10, SNAP29-5, LZTR1-16*	TOF, PDA
82	14	*TBX1-2, TBX1-7, CLTCL1-3, HIRA-25, CDC45-1, CLDN5-1, GP1BB-2, TXNRD2-9, DGCR8-2, ZNF74-2, KLHL22-2, MED15-10, SNAP29-5, LZTR1-16*	TOF, PDA
81	14	*TBX1-2, TBX1-7, CLTCL1-3, HIRA-25, CDC45-1, CLDN5-1, GP1BB-2, TXNRD2-9, DGCR8-2, ZNF74-2, KLHL22-2, MED15-10, SNAP29-5, LZTR1-16*	TOF with PA, aberrant origin of pulmonary arteries
58	15	*TBX1-2, TBX1-7, CLTCL1-3, HIRA-25, CDC45-1, CLDN5-1, GP1BB-2, TXNRD2-9, DGCR8-2, ZNF74-2, KLHL22-2, MED15-10, SNAP29-5, LZTR1-16, HIC2-2*	TOF with PA, PDA
43	15	*TBX1-2, TBX1-7, CLTCL1-3, HIRA-25, CDC45-1, CLDN5-1, GP1BB-2, TXNRD2-9, DGCR8-2, IL17RA-4, SLC25A18-1, BID-4, MICAL3-20, USP18-1, SMARCB1-1*	d-TGA, ASD, VSD
14	3	*TBX1-2, TBX1-7, SNAP29-5*	d-TGA, ASD, VSD
40	3	*TBX1-2, TBX1-7, SHANK3-22*	d-TGA, ASD, VSD, PDA

TOF: Tetralogy of Fallot, VSD: Ventricular septal defect, PA: Pulmonary atresia, d-TGA: Dextro-transposition of great arteries, ASD: Atrial septal defect, PDA: Patent ductus arteriosus