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Tetralogy of Fallot (Cyanotic Cardiac Malformation), trends and variation in a population based study

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Abstract: Congenital heart diseases (CHDs) are a major threat worldwide for children below the age of five. It has high mortality and morbidity ratio. CHDs can be classified as cyanotic and acyanotic. Cyanotic cardiac malformation accounts 25% of all congenital heart diseases and Tetralogy of Fallot (TOF) is the most common form of cyanotic CHD. Tetralogy of Fallot is a combination of four anatomical abnormalities. These include a large ventricular septal defect (VSD), right ventricular outflow tract and pulmonary valve obstruction, right ventricular hypertrophy, and over-riding of the aorta. The current investigation was conducted over the span of 2.5 years on the patients presented in OPD and hospitalized at various pediatric cardiology centers. A detailed family history was taken to elucidate the genetic and environmental factors. Diagnosis was confirmed by the cardiologist based on examination of cardiac murmur, chest X-ray, fetal echocardiography (ECG), complete blood count (CBC), echocardiograms and/or echocardiogram (ECHO) reports, cardiac catherization reports, operative notes and MRI of heart, if applicable. This study recruited a cohort of 268 patients and 140 controls, healthy unrelated individuals. The mean age for patients was 2.97±1.21 and that for controls was 3.14±2.44 years. Males were shown to have a higher rate of incidence than females (1.26: 1) in our population. The genes that are mainly involved are NKX 2.5, GATA 4, TBX5, JAG1, ZFPM2 and VEGF. The present study focuses on NKX 2.5 and its mutations in our population.

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INTRODUCTION

Congenital heart diseases (CHDs) are the most common of all birth defects and one of the leading causes of mortality in the first year of life. These figures are progressively reducing in developing countries (Figure 1), whereas increasing in underdeveloped world. CHDs can take place at any side of heart, atrial, ventricle or vascular[1]. Incidence in developing countries is currently reported to be 7-9 per 1000 live born full term births[2] (Table 1).

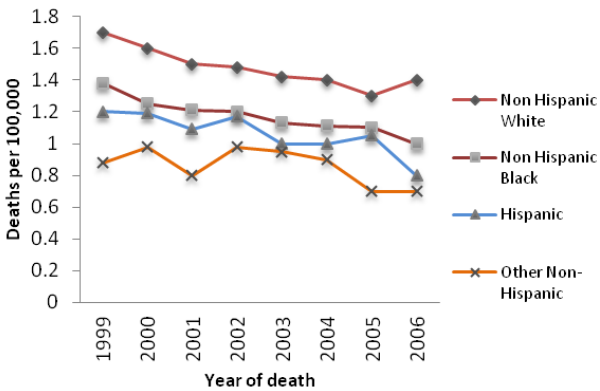

Figure 1: Graph demonstrate decline in CHD mortality in USA, CDC 2006.

CHDs can be broadly classified into two groups Cyanotic and Acyanotic. Cyanotic lesions are bluish-grey discoloration of the skin due to poor perfusion of lungs. All these account for about 25% of CHDs. Cyanotic defects include 5 T's **T**etralogy of Fallot, **T**ransposition of great arteries, **T**ricuspid atresia, **T**runcus arteriosus, **T**otal anomalous pulmonary venous connection other include Ebstein's anomaly, single ventricle and double outlet right ventricle[3].

Table 1: Incidence of CHDs.

Lesions	Frequency (Per 1000 birth)
Ventricular Septal Defects (VSD)	4
Atrial Septal Defects (ASD)	1
Atrioventicular canal (AVC)	0.3
Tetralogy of Fallot (TOF)	0.4
Dextro Transposition of Great Arteries (D-TGA)	0.2
Hypoplastic left heart syndrome (HLHS)	0.2
Double outlet right ventricle (DORV)	0.2
Pulmonary Stenosis (PS)	0.7
Patent Ductus Arteriousis (PDA)	0.8
Pulmonary Atresia (PA)	0.13
Ebstein's Anomaly (EA)	0.11
Truncus Arteriosus (TA)	0.1
Total anomalous pulmonary venous connection (TAPVC)	0.1
Tricuspid Atresia	0.1

Tetralogy of Fallot (TOF) is a congenital heart defect which is classically understood to involve four anatomical abnormalities. These are: A large ventricular septal defect (VSD), right ventricular outflow tract and pulmonary valve obstruction, right ventricular hypertrophy, and over-riding of the aorta (Figure 2). It was first described by the French physician Étienne-Louis Arthur Fallot, (1850-1911), after whom it is named[4]. The genes that are mainly involved are NKX 2.5, GATA 4, TBX5, JAG1, ZFPM2 and VEGF[5-8]. NKX 2.5, also known as CSX, cardiac specific homeobox is a transcription factor mainly used as earliest cardiac marker and crucial for

cardiogenesis. This gene is highly conserved from drosophila to humans[9,10]. The human NKX2.5 gene maps to chromosome 5q34 and consists of two exons encoding a protein of 324 amino acids. To date, more than 40 mutations within NKX 2.5 gene are reported in various congenital heart defects[11].

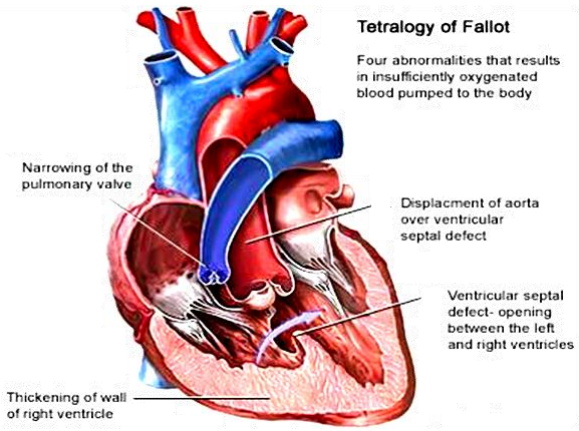

Figure 2: Tetralogy of Fallot (ADAM Medical Encyclopedia).

The etiology of TOF includes genetic and environmental factors, among these, maternal age, obesity, drugs, medication during pregnancy, lifestyle, dietary habits, maternal diabetes mellitus and consanguinity are included. In Pakistani population, cousin marriages are common and certain casts and families follow this custom as a ritual, hence increasing the risk for several inherited diseases including congenital heart diseases and conserving their genetic pool after every generation [12]. The possible risk factor is increased 2-11% in first degree relatives than general population [13]. The common type of consanguineous marriages and their coefficient of inbreeding are listed in table 2.

Table 2: Coefficient of inbreeding.

Type	Degree of Relationship	Population of Gene in common	Coefficient of Inbreeding of offspring
Double first cousin	2nd	1/4	1/8
First cousin	3rd	1/8	1/16
Half Uncle Niece	3rd	1/8	1/16
Half first cousin	4th	1/16	1/32
Second cousin	5th	1/32	1/64

The present study demonstrates frequency of this disease; with its variation in our population and also determines some genetic and environmental factors like consanguinity and mutations.

MATERIALS AND METHODS

The study, after formal approval by the respective Institutional Review Board, was conducted in various pediatric cardiology centers of Karachi, over the span of 2.5 years (Jan'2007- July'2009). The patients were recruited after the confirmation of pediatric cardiologist and examining the cardiac murmur, chest X-ray, fetal echocardiography (ECG), complete blood count (CBC), echocardiograms and/ or echocardiogram (ECHO) reports, cardiac catherization reports, operative notes and MRI of heart. Patients with syndromes associated with congenital heart disease like Down syndrome, Marfan, Noonan, Digeorge syndrome were excluded. A cohort of 268 patients and 140 controls, healthy unrelated individuals were selected. All patients were sporadic and non syndromic. A detailed family history was taken in order to elucidate the consanguinity status of marriage and relationship among couples as well as mothers were interviewed for their dietary, medication, infection (rubella) status and diabetes mellitus during pregnancy. After informed consent was taken from the parents or guardians of the patients, blood samples were taken from the patients in ACD vacutainers. DNA extraction was done by phenol chloroform isoamyl alcohol method. NKX 2.5 gene was amplified with four set of primers to cover entire exon-intron boundaries[6]. Statistical analyses were done by SPSS 17.0 version.

RESULTS

There were 153 males and 115 females patients with a mean age of 2.97±1.21 and that for controls was 3.14±2.44. The age ranges from one day to 12 years of age and shows normal distribution with a significant p value (<0.001). The weight for patients' means was 12.30±6.62 and showed normal distribution with slight right-skeweness (Figure 4). Males were shown to have a higher rate of incidence than females in our population. Various categories of Tetralogy of Fallot (53.73%) and its association with other forms were found to be; pulmonary atresia (5.22%), patent ductus arteriosis (7.46%), pulmonary stenosis (4.47%), tet spells (2.23%), left to right shunt (4.85%), large atrial septal defect II (3.35%), right S/P shunt (3.73%), ASD with R-L shunt (4.10%), Patent foramen ovule with L-R shunt (4.47%%), dextrocardia (1.49%), total correction (1.49%), pulmonary stenosis and ventricle septal defect (1.86%), ASD II with L-R shunt and patent ductus arteriosus (1.49%) (Table 3).

A significant association between cousin marriages and Tetralogy of Fallot was found with chi square test ($p<0.001$).

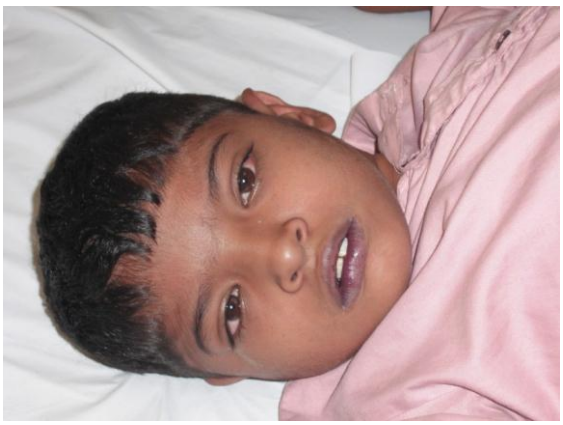

Figure 3: Patient with Tetralogy of Fallot, cyanosis is visible at lips and eyes.

Two mutations were found in the patients, one at codon 22 Q to P already reported. In coding region CAG to CCG, mutation was found at the second position of the codon[6,14] and other mutation in the patients with TOF and ASD A to T at codon 135 was also reported. The change occurs at 1st position of codon from GCG to ACG[15].

Table 3: Tetralogy of fallot and its variation.

Types	Male	Female	Total	Percentage
Tetralogy of fallot	85	59	144	53.73
TOF with PA	7	7	14	5.22
TOF with PDA	13	7	20	7.46
TOF with PS	10	2	12	4.47
TOF with tet spells	4	2	6	2.23
TOF with L-R shunt	6	7	13	4.85
TOF with large ASD II	6	3	9	3.35
TOF with right S/P shunt	3	7	10	3.73
TOF ;ASD, with R-L shunt	5	6	11	4.10
TOF, PFO with L-R shunt	5	7	12	4.47
TOF with dextrocardia	2	2	4	1.49
TOF with TC	2	2	4	1.49
TOF, PS, VSD	3	2	5	1.86
TOF with ASD II, L-R shunt and PDA	2	2	4	1.49
	153	115	**268**	

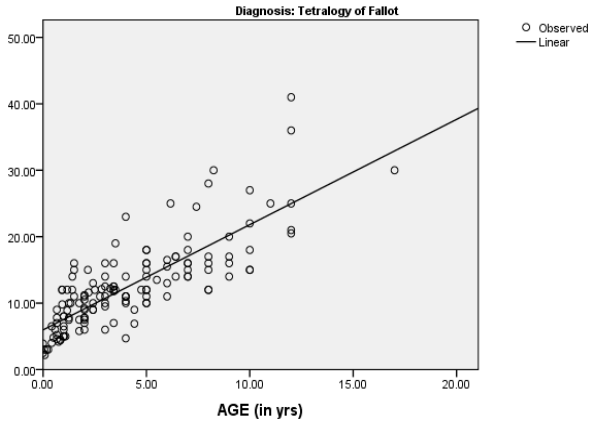

Figure 4: Showing weight distribution for the patients.

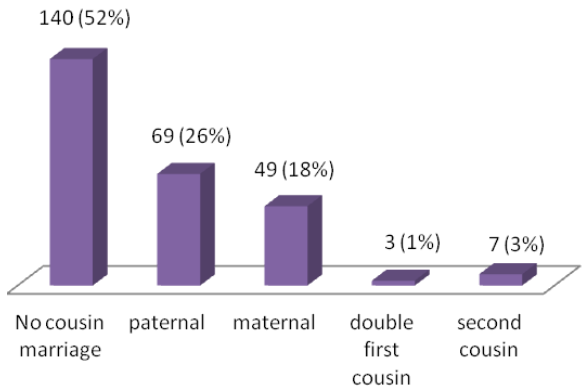

Figure 5: Frequencies of cousin marriages among TOF patients and its percentages.

DISCUSSION

The incidence of different types of congenital heart diseases per 1000 births indicates that VSD is the most common abnormality and tricuspid atresia is the highly rare malformation in the congenital heart diseases. Anatomical variation has a wide spectrum, higher rates observed with pulmonary artery obstractions, atresia and stenosis (10%). TOF with septal defects ASD, VSD, ASD II are collectively (8%)[16].

It is clear from the data, that consanguinity affects the rate of congenital heart disease, as it is 48% in diseased group and 28% in control group (Figure 5). Similar pattern for consanguinity, cousin marriage has been reported in Saudi and Jordanian populations[17,18].

NKX 2.5 gene has ensemble gene ID ENSG00000183072 and transcript ID 00000329198, Uniprot ID P52952. The first mutation that took place at 65bp of coding region, codon 22 assessed with mutation tester predicts that it is a disease causing mutation with a score of 2.07. There is a change from Q to P, Glutamic acid to Proline, from polar to a non-

polar amino acid. The second mutation was at 403bp changing A to T, Alanine to Threonine, from a non-polar amino acid to polar amino acid with a score of 2.92, predicting a polymorphism with 0.98 probabilities.

In developing countries, the rate of mortality due to congenital heart disease is decreasing and number of adult population with CHD is increasing due to advancements and improved quality of clinical facilities while in this country/region the situation is inverse. General public awareness should be developed and immediate medical care should be provided to such suffering little angles.

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