CHROMOSOME 22q11 DELETION IN PATIENTS WITH SELECTED CONGENITAL HEART DISEASE (CONOTRUNCAL MALFORMATIONS): STUDY OF PREVALENCE, PHENOTYPE, GENOTYPE AND MODE OF INHERITANCE

SYNOPSIS OF THESIS

Submitted by

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Registration number: KFM04DP001

FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN FACULTY OF ALLIED HEALTH SCIENCES

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October 2013
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Chapter 1
Prevalence of chromosome 22q11 deletion in conotruncal defects

INTRODUCTION
The 22q11 region has been associated in chromosomal rearrangements that lead to altered gene dosage, resulting in different congenital malformations. It is estimated that almost 10% of the present infant mortality in India may be due to congenital heart disease. The chromosome 22q11 deletion is mainly characterized by congenital cardiac defect mainly conotruncal heart defect (CTHD), aplasia or hypoplasia of parathyroid, cleft palate, hypocalcemia and facial abnormalities. Various reports have shown differences in the prevalence of chromosome 22q11 deletion in CTHD. The data on frequency of chromosome 22q11 deletion in specific subtypes of CTHD is limited, hence, this study was carried out on Indian children with different lesions of conotruncal defects.

AIMS:
1. To determine the frequency of chromosomal aberrations particularly chromosome 22q11 deletion in Indian children ≤ 2 years with different types of conotruncal malformations and their association with abnormal aortic arch.
2. To detect the frequency of chromosomal aberrations in parents of children with chromosome 22q11 deletion.

REVIEW OF LITERATURE:
Congenital heart defect (CHD) is a common birth defect affecting approximately 0.8% of all live births. The incidence of chromosome deletion syndrome (22q11DS) is estimated to be 1 in 4,000 live births. The reduced gene dosage on 22q11 results in DiGeorge, velocardiofacial and conotruncal anomaly face syndrome. The cytogenetic and molecular cytogenetics techniques have led to considerable progress in understanding role of genetics in such defects.

METHODS:
In the present study, 254 consecutive live-born children with 2 years or less than 2 years of age were recruited for the study. Conventional cytogenetic and fluorescence in situ hybridization (FISH) analysis were performed in all patients with conotruncal defects. The
seven types of conotruncal defects were categorized as: tetralogy of Fallot (TOF), tetralogy of Fallot with pulmonary atresia (TOF / PA), tetralogy of Fallot with absent pulmonary valve (TOF / APV), double outlet right ventricle (DORV), conoventricular ventricle septal defect (CVVSD), truncus arteriosus (TA) and interrupted aortic arch (IAA). The relation between 22q11 deletion and demographic, auxological parameters were also studied. Parental screening for deletion status was performed wherever possible.

RESULTS:
The present study reveals chromosomal abnormalities in 52 (21%) children with conotruncal defect, including 49 (94%) patients with 22q11 deletion (Fig 1) and three (6%) had other abnormalities (Fig 2). The chromosome 22q11 deletion was detected in 19% (49/254) of children with conotruncal malformations.

![Figure 1: FISH analysis showing deletion of DiGeorge/VCFS (TUPLE1) region on chromosome 22q11.2 indicated by absence of red signal on one of the chromosome in metaphase spread and nucleus.](image1)

![Figure 2: Karyotype 46,XY, del(6)(q14q21) in a child with CVVSD](image2)

The frequency of deletion varied in subtypes of CTHD, maximum been 50% in 8 patients with interrupted aortic arch whereas no deletion found in 11 patients with TOF/APV. In addition the association of deletion with arch sidedness also varied with the type of
conotruncal defect (Table 1). The additional finding of right aortic arch in present series of children with CTHD has increased the risk for 22q11 deletion in TOF (P =0.021) and TOF/PA (P =0.027) and may be truncus arteriosus (small sample size) but not in other types of defects. Cytogenetic analysis showed normal karyotype in all patients, except three cases (Table 2). In two cases, maternally derived deletion was observed.

Table 1. Frequency of 22q11 deletion and arch sidedness in conotruncal defects

<table>
<thead>
<tr>
<th>Conotruncal defect</th>
<th>Deleted patients - percentage (deleted/total)</th>
<th>Frequency</th>
<th>Right arch</th>
<th>Left arch</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetralogy of Fallot</td>
<td>15.1(16/106)</td>
<td>27 (10/37)</td>
<td>8.7 (6/69)</td>
<td>0.021*</td>
<td></td>
</tr>
<tr>
<td>TOF with pulmonary atresia</td>
<td>-</td>
<td>-</td>
<td>14.6(6/41)</td>
<td>0.027*</td>
<td></td>
</tr>
<tr>
<td>TOF with APV</td>
<td>-</td>
<td>-</td>
<td>0 (0/11)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Double outlet right ventricle</td>
<td>13.3(4/30)</td>
<td>0 (0/7)</td>
<td>23.2(13/56)</td>
<td>46.7(7/15)</td>
<td></td>
</tr>
<tr>
<td>Conoventricular VSD</td>
<td>20 (6/30)</td>
<td>0 (0/7)</td>
<td>0 (0/11)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Truncus Arteriosus</td>
<td>46.2 (6/13)</td>
<td>60 (3/5)</td>
<td>37.5(3/8)</td>
<td>0.592(NS)</td>
<td></td>
</tr>
<tr>
<td>Interrupted aortic Arch</td>
<td>50.0 (4/8)</td>
<td>-</td>
<td>50.0(4/8)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19.3 (49/254)</td>
<td>28.2 (20/71)</td>
<td>15.8(29/183)</td>
<td>0.026*</td>
<td></td>
</tr>
</tbody>
</table>

TOF = Tetralogy of Fallot; VSD = Ventricle septal defect; APV = Absent pulmonary valve; *p < 0.05; NS = Non significant.

TABLE 2. Chromosomal abnormalities besides 22q11 deletion

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Karyotype</th>
<th>22q11 deletion</th>
<th>Heart defect</th>
<th>Arch sidedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46,XY,del(6)(q14q21)</td>
<td>No</td>
<td>DORV, CVVSD</td>
<td>Left</td>
</tr>
<tr>
<td>2</td>
<td>46,XX,del(2)(q34-qter)</td>
<td>No</td>
<td>TOF/APV</td>
<td>Left</td>
</tr>
<tr>
<td>3</td>
<td>45,XO</td>
<td>No</td>
<td>CVVSD</td>
<td>Right</td>
</tr>
</tbody>
</table>

TOF = Tetralogy of Fallot; APV = Absent pulmonary valve; DORV = double outlet right ventricle, CVVSD = Conoventricular ventricle septal defect.

DISCUSSION

This is first major study performed in India evaluating the frequency of chromosome 22q11 deletion in 254 children particularly ≤ 2 years of age with conotruncal defects. The frequency
of 22q11 deletion in conotruncal malformations is found to vary between 7% - 48%,\textsuperscript{1,2} it may be due to the patient selection criteria - based on type of cardiac defect, age of diagnosis and the techniques used. The children up to 2 years of age with conotruncal defects were chosen because this is the most common age for presentation of conotruncal malformations and subtle phenotypic features as compared to older children and adults.

The reported frequency of 22q11 deletion in TOF/APV is high,\textsuperscript{2} however no deletion was identified in our series of 11 patients. The 22q11 deletion is rarely associated with DORV, but present data indicates a comparatively high frequency (13%) of deletion. The knowledge of 22q11 deletion is important based on the type of cardiovascular lesion, since the surgical prognosis in patients with deletion varies with the type of conotruncal defects.\textsuperscript{3}

Although the frequency of deletion was significantly high in patients with right arch as compared to normal left arch, there was difference in its prevalence based on the subtypes of conotruncal defects. A number of low copy repeats (LCR) present in the 22q11.2 region plays a major role in the 22q11 deletion syndrome (22q11 DS). The presence of other chromosomal aberrations besides 22q11 microdeletion in three patients in this study, specify a need of conventional cytogenetic analysis in conotruncal malformations.

In the current study, two cases showed maternally derived deletion. The mother with deletion and normal father in case of child with TOF confirms mode of inheritance as an autosomal dominant. In addition, the variable feature in the mother and child with deletion shows absence of phenotype genotype correlation.

**CONCLUSION**

The study suggests that specific type of conotruncal defect and associated cardiovascular anomaly should alert the clinicians for 22q11 deletion testing. The screening of chromosomal anomalies at an early age assists in better management of patients, thus preventing severe complications. It highlights the need for studying both parents when a child is found to have a deletion, since such findings has implications for genetic counseling. The study also indicates a need for further research in order to understand the basis for variation in frequency of 22q11 deletion in subtypes of conotruncal defects.

**REFERENCES:**
Chapter 2

Phenotypic predictors of chromosome 22q11 deletion

INTRODUCTION
More than 180 phenotypic features are found associated with 22q11 DS, including congenital heart disease, mainly conotruncal malformations and craniofacial defects. Although classical phenotypic features of 22q11 DS are shown in the literature, but due to the striking variability in the clinical expression and absence of genotype phenotype correlation in patients with 22q11 DS, the identification is a diagnostic challenge. However the phenotypic predictors of deletion can be useful for patient management and counseling.

AIM
To find the phenotypic predictors of deletion which can help clinicians to provide more appropriate interventions in case of unavailability of genetic facilities?

REVIEW OF LITERATURE
Patients with 22q11 DS have a wide spectrum of clinical features (180 features) including CHD and characteristic facial features. The correlation between phenotype and genotype is not found since the phenotype is highly variable.¹ Many patients are undiagnosed because of few signs and symptoms and due to expensive techniques to detect such deletions.

METHODS
The data on extracardiac features were available for 226/254 children. It included systematic physical examination and clinical history. Univariate and multivariate logistic regression analyses were performed to ascertain extracardiac features (16 physical features and hypocalcemia) helpful in identifying high-risk patients with chromosome 22q11 deletion.
RESULTS
The mean birth weight was 2.58 ± 0.36 Kg in deleted group as compared to 2.93 ± 0.53 Kg in congenital heart defect. The genotype phenotype correlation showed that specific extracardiac features were more common in children with deletion as compared to those without deletion (Fig 1). The multivariable logistic regression analysis illustrate that eight extracardiac features in combination had 93.5% agreement with the presence of deletion by FISH test (Table 2). All 44 patients with monosity of 22q11 showed one or more extracardiac features of 22q11 DS.

![Figure 1 Comparison of extracardiac features in deleted and nondeleted groups](image)

Figure 1 Comparison of extracardiac features in deleted and nondeleted groups

Table 2: Extracardiac features found significant by multivariate analysis

<table>
<thead>
<tr>
<th>Feature</th>
<th>p Value</th>
<th>Odd’s Ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low set ears</td>
<td>0.001</td>
<td>12.73</td>
<td>3.01 – 53.8</td>
</tr>
<tr>
<td>Dysplastic flared pinnae</td>
<td>&lt;0.001</td>
<td>20.9</td>
<td>4.4 – 99.7</td>
</tr>
<tr>
<td>Short palpebral fissures</td>
<td>0.002</td>
<td>11.75</td>
<td>2.5 – 55.3</td>
</tr>
<tr>
<td>Square nasal tip</td>
<td>0.003</td>
<td>10.3</td>
<td>2.2 – 49.0</td>
</tr>
<tr>
<td>Microstomia</td>
<td>&lt;0.001</td>
<td>21.9</td>
<td>4.1 – 119.1</td>
</tr>
<tr>
<td>High arched palate</td>
<td>0.027</td>
<td>4.4</td>
<td>1.2 – 16.6</td>
</tr>
<tr>
<td>Thin long fingers</td>
<td>0.026</td>
<td>4.42</td>
<td>1.2 – 14.8</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>&lt;0.001</td>
<td>33.2</td>
<td>6.0 – 183.9</td>
</tr>
</tbody>
</table>

DISCUSSION
The clinical prediction of 22q11 deletion syndrome is difficult due to phenotypic variability. Owing to limited genetic centres and expensive FISH technique, chromosome 22q11 deletion testing in all patients with conotruncal defects is not possible in developing and underdeveloped countries. Hence the eight extracardiac features can be helpful in two
conditions, firstly the extracardiac features can be used as predictors of deletion and hence FISH test can be ordered in such cases, secondly, in taking clinical decision pertaining to specific perioperative interventions (irradiated blood transfusions), counselling family and in order to manage the associated complications of 22q11 DS. The study shows that these eight extracardiac features may add to the expansion of the phenotypic criteria used in screening of patients at risk for 22q11 microdeletion.

CONCLUSIONS

The study suggests screening of chromosome 22q11 deletion in specific types of conotruncal defects. However, if deletion analysis is not possible, specific extracardiac features (six dysmorphic facial features, thin long fingers and hypocalcemia) can help to identify an increased risk of 22q11 deletion in patients with conotruncal defect especially in limited resource settings.

REFERENCES


Chapter 3

Mean platelet volume and platelet count in children with or without chromosome 22q11 deletion

INTRODUCTION

A wide range of clinical features including megathrombocytopenia is found to be associated with 22q11 DS. There is no study showing association between Mean platelet volume (MPV) and chromosome 22q11 deletion in patients with conotruncal defects from India. Although few studies have shown megathrombocytopenia in deleted patients with DiGeorge syndrome, but the association of these features with different types of conotruncal defects is not well defined.

AIM

Our aim was to compare Mean platelet volume and Platelet count between deleted and nondeleted group with conotruncal heart defects.
REVIEW OF LITERATURE

A wide range of clinical features associated with 22q11 DS makes the clinical diagnosis of 22q11 deletion challenging. An increased MPV has been found to be a predictor of chromosome 22q11 deletion. In 22q11 DS, thrombocytopenia is found to be associated with decreased expression of glycoprotein Ib-beta due to deletion of chromosome 22q11.

METHODS

In a retrospective study, microdeletion of chromosome 22q11 and platelet parameters were observed in 193 children with conotruncal defects. The MPV and platelet count in 39 patients with 22q11 deletion were compared with 154 cases without deletion.

Chi-square test was done to find the statistical significance of the difference in percentage of platelet parameters between deleted and nondeleted group. Besides these, the receiver operating characteristic curve for MPV was also performed. The mean of platelet count and MPV were analyzed by independent T test. The correlation coefficient between MPV and platelet count in both groups was also found.

RESULTS

In deleted versus nondeleted groups, the mean platelet volume was 10.5 ± 2.5 fL vs. 7.6 ± 1.5 fL and platelet counts was found to be 225 ± 80.7 vs. 339 ± 127.3 x10^9 /l. In deleted versus nondeleted patients, the frequency of increased MPV was 49% vs.7% (p <0.0001), while the frequency of thrombocytopenia was 16% vs.7% (p=0.11). A non significant negative correlation was found between MPV and platelet count in deleted group. Based on the subtypes of conotruncal defects the frequency of high MPV in deleted patients varied. In case of TOF/APV, neither deletion nor increased MPV was observed.

The area under receiver operating characteristics (ROC) curve for MPV in predicting 22q11 deletion in patients with conotruncal defect was found to be 0.91 (95% CI 0.86 to 0.96) (p <0.001). Using the ROC curve, the best cut-off value for MPV was found to be 8.32 fL, with a sensitivity of 90.9% and specificity of 79.6%.

DISCUSSION

The strong association between MPV and chromosome 22q11 deletion suggests that elevated MPV is an additional potential useful phenotypic marker for chromosome 22q11 deletion. A probable cause for macrothrombocytopenia is platelet genesis depends on the GPIb/V/IX complex-membrane density and that a deficiency of this complex leads to bigger and thus less platelets. A high area under ROC curve of MPV indicates that it can be useful predictor
of chromosome 22q11 deletion. The cut-off value of 8.32 fL may be used as an indicator of high risk of 22q11 deletion.

However our study revealed a non significant (p=0.11) association between low platelet count and deletion, on the contrary, VanGeet et al reported a significant negative correlation in 38 deleted patients.2 Thrombocytopenia was found in patients with hemizygosity of chromosome 22. The study also shows that the frequency of elevated MPV was significantly high in deleted children with severe form of conotruncal defects like interrupted aortic arch, TOF/PA as compared to less severe type like tetralogy of fallot, but more numbers are needed to prove it.

**CONCLUSIONS**

Our results suggest that mean platelet volume above 8.3 fL can be an indicator of high risk of chromosome 22q11 deletion in patients with conotruncal defects. The cause of low platelet count may not be 22q11 deletion but some secondary factors may be responsible for it. We believe that more extensive study is needed in order to understand the disparity in frequency of high MPV in deleted patients with different lesions of conotruncal defects.

**REFERENCES**


**Chapter 4**

**Screening for TBX1 gene in children with or without microdeletion of chromosome 22q11 and conotruncal defect**

**INTRODUCTION**

The chromosome 22q11.2 region contains nearly 30 well defined genes including TBX1. It is noticed that loss of Tbx1 gene is responsible for the cardiovascular abnormalities in mice.

**AIM**

Screening for TBX1 gene in children with or without microdeletion of chromosome 22q11 and conotruncal defect.
REVIEW OF LITERATURE
T-Box genes are transcription factors involved in the regulation of developmental processes. TBX1 is found to be candidate gene for the critical regulator of outflow tract development in DiGeorge syndrome. In a study carried out on 558 patients with 22q11 DS the result showed the common 3Mb or 1.5 Mb proximal nested deletion including the TBX1 within 22q11.2 region in 37 of 200 (18.5%) patients with conotruncal heart defects.1

MATERIALS AND METHODS
The 22 patients randomly selected with conotruncal defects comprised of two groups, group I had ten patients with deletion and group II had 12 patients without deletion for TUPLE1 locus on chromosome 22q11. These patients were screened for DiGeorge critical region by FISH technique using commercial probe TBX1 (Cytocell) and N25 (Vysis) on chromosome 22q11.

RESULTS
In case of children tested with multiple probes, in group I all ten patients with deletion of TUPLE1 region were also found to be deleted for TBX1 and N25 loci on chromosome 22q11. In group II, all 12 patients without deletion for TUPLE1 locus, showed normal FISH signals with TBX1 and N25 probe. None of the patients had hypothyroidism.

DISCUSSION
The ten children in group I deleted for TUPLE1 locus were also found to be deleted for TBX1 locus, since TBX1 gene is suggested to be one of the candidate gene responsible for DiGeorge syndrome. The advantage of using TBX1 probe is that it can detect deletion of the TBX1 locus, which can be missed by routinely used TUPLE1 or N25 probes. In group II, all 12 patients without deletion for TUPLE1 locus, showed normal FISH signals with TBX1 and N25 probe which indicates that etiology of conotruncal defects is multifactorial.

CONCLUSION
Owing to small sample size it is difficult to draw conclusions, however, the study confirms that contiguous genes including TBX1 are involved in the etiology of CTHD. However, a more detailed study on the mechanism of interaction between multiple genes is important in order to better understand the etiology behind conotruncal malformations.

REFERENCES