CHAPTER 5

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

It is observed that interstitial deletion of chromosome 22q11 is often associated with conotruncal cardiac defects. In CHAPTER 1, the frequency of chromosomal aberrations particularly chromosome 22q11 deletion in 254 Indian children ≤ 2 years of age with different types of conotruncal malformations and their association with abnormal aortic arch are presented. Besides these, familial inheritance of chromosomal aberrations was also detected. In CHAPTER 2, the phenotypic predictors of chromosome 22q11 deletion are shown. In CHAPTER 3, the mean platelet volume and platelet count in children with and without chromosome 22q11 deletion were noticed. In CHAPTER 4, TBX1 gene was screened in children with or without microdeletion of chromosome 22q11 and conotruncal defect. In addition few children were also tested for DiGeorge critical region II on short arm of chromosome 10.

5.1 CHROMOSOME 22q11 DELETION IN CONOTRUNCAL DEFECTS

The literature survey showed variations in the reported prevalence rate for chromosome 22q11 deletion between 2%-18% in conotruncal malformations. However, there are limited studies showing frequency of microdeletion in different subtypes of conotruncal cardiac defects. This thesis work has helped us to know the prevalence rate of chromosome 22q11 deletion in a large sample size of 254 Indian children with conotruncal defects. This is the first large study carried out to detect microdeletion of chromosome 22q11 in children, specifically 2 years or less than 2 years of age with conotruncal heart defects. A frequency of 19.3% of interstitial deletion of chromosome 22q11 region was noticed in present cohort of patients. It indicates a major role of genomic changes in the etiology of conotruncal cardiac defects. The 22q11 DS is a rare genetic disorder with a prevalence rate of 1 per 4000 live births. Despite this, we were able to detect 49
children with 22q11 deletion in a short duration of time from a single tertiary care centre. However, review of literature shows that the frequency of 22q11 deletion does not vary much based on geographical location.

Previous studies have showed high frequency of deletion between 21% - 75% in Tetralogy of fallot with absent pulmonary valve, a type of conotruncal defect [9,205], on the contrary, no deletion was identified in this study. This finding begs the question of whether 22q11 deletion in TOF/APV is rare in Indian children or it is by chance that none of the patients with deletion were found. However, only a small number of studies were carried out to find frequency of deletion in Tetralogy of Fallot with absent pulmonary valve. Earlier studies have shown that the chromosome 22q11 microdeletion is rarely linked with double outlet right ventricle, but this work shows a comparatively high frequency of deletion. Hence new studies with large sample size in such types of conotruncal defects are warranted in order to understand exact frequency of 22q11 deletion. With such studies, possibly the frequency of association of specific type of conotruncal defects with chromosome 22q11 deletion will prove to be helpful for the clinicians and therefore more valid to genetic research.

In the present work, the right arch sidedness was found to be more frequent than normal left arch in patients with microdeletion, which was in accordance to few other studies. Although the frequency of deletion was significantly higher in patients with right arch as compared to normal left arch, there was difference in its frequency based on the subtypes of conotruncal defect. The additional finding of right aortic arch in the present series of children with conotruncal defect, has increased the risk for 22q11 deletion in TOF, TOF/PA and may be truncus arteriosus but not in other types of defects. Therefore, right aortic arch in specific types of conotruncal defects can help in identifying an increased risk of chromosome 22q11 deletion in individuals with conotruncal defects, hence such patients should undergo deletion testing.

The main advantage of FISH technique includes high sensitivity, specificity and ease. It is able to detect microdeletion of chromosome 22q11.2 region in 96% of cases with 22q11 DS. It identifies submicroscopic genomic changes undetected by classical cytogenetic technique. It was observed that
availability of genetic testing facilities resulted in significant increase in frequency of diagnosis of DiGeorge syndrome and Velocardiofacial syndrome, hence it is believed that such genetic diagnosis resulted in increased referral which in turn is useful for patient management and care. This work emphasizes the significance of cytogenetic screening of a high-risk group of the patients for 22q11.2 microdeletion.

The study suggests that specific type of conotruncal defect and associated cardiovascular anomaly should alert the clinicians for 22q11 deletion testing. In addition, screening of chromosomal anomalies at an early age will assist in better management of patients, thus preventing severe complications. The presence of other chromosomal aberrations besides 22q11 microdeletion, specify a need for conventional cytogenetic analysis in addition to FISH in individuals with conotruncal malformations. A question that should be further explored is the basis for variation in frequency of 22q11 deletion in different subtypes of CTHD.

5.2 PHENOTYPIC PREDICTORS OF 22q11 DELETION

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, the clinical diagnosis of 22q11 deletion is a challenge. It is likely that considerable number of individuals with deletion remains unrecognized due to unavailability of genetic testing facilities especially in developing and underdeveloped countries. FISH analysis on metaphase cells is time consuming, expensive as well as a labour-intensive process. Therefore, in such conditions, the phenotypic predictors of deletion can be useful for management of individuals with conotruncal defects. The present work identified eight phenotypic features that could potentially predict chromosome 22q11 deletion. These eight extracardiac features (six dysmorphic facial features, thin long fingers and hypocalcemia) may be helpful in two conditions, firstly the extracardiac features may be useful to identify an increased risk of 22q11 deletion in patients with conotruncal defect and hence FISH test can be ordered in such cases, secondly, in taking clinical decision pertaining to specific perioperative interventions (irradiated blood transfusions), family counselling and to manage the
associated complications of 22q11 deletion syndrome, even if advanced
techniques like microarray, MLPA and FISH techniques are unavailable.

The extracardiac features identified from a group of clinical features
often associated with 22q11 DS may add to the expansion of the phenotypic
criteria used in screening of patients at risk for 22q11 deletion. The ability of the
phenotypic markers identified to prospectively predict the presence/absence of
22q11 microdeletion needs to be validated. The phenotypic markers can help to
indicate high risk of 22q11 deletion, but confirmation is possible only with genetic
study. Though it cannot replace cytogenetic testing, these observations can have
wide-ranging clinical application in situations where either FISH testing is
unavailable or in conditions where need for an emergency intervention does not
permit the time for cytogenetic diagnosis.

5.3 Platelet parameters

The study confirms that MPV can be an easy, time-saving and cost effective
screening marker to make a decision on when to perform the deletion testing in
case of syndromic conotruncal defects. The frequency of MPV was found to be
high in individuals with deletion and severe form of conotruncal defects, but
studies with large sample size is required to understand the actual frequency and
detailed studies should be performed to understand the cause for this.

The present results postulates the best cut-off value for MPV to be 8.32 fL,
with a sensitivity of 90.9% and specificity of 79.6%, hence, MPV above 8.32 fL
can be an indicator of high risk of 22q11 deletion in patients with conotruncal
defects. In case of unavailability of genetic diagnosis, this parameter could
possibly assist high risk patients suspecting deletion for an emergency
intervention. This simple haematological parameter MPV can facilitate the
clinicians for better follow up, since a multifaceted approach is necessary in
children with hemizygosity of chromosome 22q11 region. The thrombocytopenia
is found in patients with 22q11 DS, the cause of which may not be deletion of
chromosome 22q11.2 region but some secondary factors may be responsible for it.
Prospective studies with large sample size are needed to evaluate the cause of low
platelet count in 22q11 deletion syndrome.
5.4 *TBX1* GENE AND CONOTRUNCAL DEFECTS

Various studies have witnessed an accelerated attempt to understand the role of genetics in conotruncal cardiac defects, but no specific gene(s) is found to be responsible for a particular phenotypic feature in 22q11 DS. This research work was useful to understand the role of *TBX1* gene in children with or without 22q11 deletion and conotruncal defect. The advantage of using TBX1 probe is that it can detect deletion of TBX1 locus which can be missed by routine TUPLE1 or N25 probes. Since multiple genes are involved in the etiology of this syndrome, so in routine clinical practice, FISH probes TUPLE1, N25 and TBX1 can be used to detect 22q11 deletion in conotruncal defects.

In group I, testing with multiple probes demonstrated that deletion size was more than minimum DiGeorge critical region. These data together with the conotruncal defects observed in various mice with loss of *Tbx1* gene suggest that the *TBX1* is a promising candidate gene for conotruncal heart defects.

However in group II, all 12 patients without deletion for TUPLE1 and N25 loci, showed normal FISH signals with TBX1, indicating that the etiology of conotruncal defects is multifactorial. Nevertheless there is still lack of knowledge concerning the exact role of *TBX1* gene in Human. Owing to small sample size of 22 patients in this part of study, it is not appropriate to draw conclusions, however, it provides useful insight into further research especially with regards to role of *TBX1* gene in conotruncal defects in human.

The results of such genetic testing is important for clinical decision makers, since patients with 22q11 deletion need early medical intervention based on associated symptoms. However, there are still numerous gaps in our knowledge concerning the relation between genomic variations and conotruncal cardiac defects, which, hopefully, will be filled by forthcoming studies.