CHAPTER 3
MEAN PLATELET VOLUME AND PLATELET COUNT IN
CHILDREN WITH OR WITHOUT CHROMOSOME 22q11
DELETION

3.1 INTRODUCTION

Congenital heart defect is one of the main causes of high mortality and morbidity in infants. A wide range of clinical features (more than 180) including conotruncal heart defect and megathrombocytopenia (platelets of increased size and thrombocytopenia) are found to be associated with chromosome 22q11 deletion syndrome, hence the clinical diagnosis of 22q11 deletion is not so easy. It is observed that mean platelet volume, an indicator of platelet function detected by simple routine blood test can be a useful predictor of chromosome 22q11 deletion [382].

3.1.1 PLATELETS

Platelets are tiny non nucleated disc-shaped cells (2µm diameter) that circulate in the blood and whose function is to take part in the clotting process. They are produced in the bone marrow from megakaryocytes. The platelets circulating in the blood show variations in size and haemostatic potential [383-384]. The platelet count is found to be constant in EDTA (Ethylenediaminetetraacetic acid) anticoagulant. Platelets stored in citrate are significantly smaller in contrast to those stored in EDTA [385]. Thrombocytopenia is the term for a reduced platelet (thrombocyte) count. A normal platelet count ranges from 150 - 450 x10^9 /l of blood, platelet count below 50,000 per microliter results in thrombocytopenia [382]. Both increase and decrease of platelet counts result in condition of excess bleeding or clotting. Low platelet counts can be attributed to a number of disease processes including 22q11 DS [387].
Mean platelet volume

Mean Platelet Volume (MPV) is a measurement of the average size of platelet found in blood and can be measured by automated hematology analyzer. The MPV is a useful surrogate marker of platelet function. Normal MPV range is approximately 7 to 10 fL (femtoliters). Several studies have shown both high and low values of MPV associated with a variety of cardiovascular and inflammatory disorders. The platelet volume measurement depends on time, optimal measuring time should be within 120 minutes after venipuncture [385].

The platelet size is regulated by multiple factors. The MPV can give an idea about platelet turnover, since newly released platelets from the bone marrow are larger and have a tendency to decrease in size with age in the circulation [388]. In congenital macrothrombocytopenias typically large platelets are observed, which is at least twice the normal size and perhaps as large as erythrocytes. The MPV value can help to explain the cause of thrombocytopenia. During megakaryocytopenesis, platelet size is chiefly determined in the bone marrow, and subsequently does not substantially change. The MPV is regulated only to a degree by thrombopoietin. Actually growth factors and cytokines may also be responsible for the production of larger and more reactive platelets in the bone marrow [389]. In conditions like obesity, endothelial dysfunction and possibly myocardial and cerebral ischaemia, there is chance of increase in concentrations of cytokines, which results in increased MPV [390-394]. A significantly high value is also found in patients with immune thrombocytopenic purpura. This simple laboratory test showing MPV along with clinical features may help in knowing about cardiovascular and cerebrovascular risk, and aid in giving information about unexplained thrombocytopenia.

3.1.2 22q11 DS AND PLATELETS PARAMETERS

4. Few studies have associated megathrombocytopenia with chromosome 22q11 deletion in patients with DiGeorge syndrome [354,387]. The Glycoprotein (GP) Ib-IX-V complex, a platelet adhesion receptor is found to play a significant role in thrombin aggregation and activation [395,396]. It is formed by the assembly of four transmembrane
proteins, separately encoded by genes GPIbα on 17p12, GPIbβ on 22q11.2, GPV on 3q29 and GPIX on 3q21 [397-403]. For efficient biosynthesis of the receptor all four genes are required [404]. Bernard-Soulier syndrome (BSS), a recessive disorder usually caused by a homozygous GP Ibα gene dysfunction, results in decreased or absent GP Ib/IX/V complex on the platelet membrane, and characterized by thrombocytopenia and enlarged platelets [405]. The GPIbβ gene is mapped on the chromosome 22q11.2 [400], hence the patients with a 22q11 deletion are obligate heterozygous carriers for the gpIb-beta deletion and therefore, are heterozygotes for BSS. Budarf et al. have showed a case study of a boy with chromosome 22q11 deletion and BSS [406].

5. In 22q11 DS, thrombocytopenia is found to be associated with decreased expression of glycoprotein Ib-beta gene, due to deletion of chromosome 22q11 region [387]. The study by Levy et al. reports about two female patients carriers of chromosome 22q11 deletion showed thrombocytopenia. In this one of the patients had idiopathic thrombocytopenic purpura, whereas the reason for thrombocytopenia in the second patient was unknown [407]. The patients with 22q11 DS and thrombocytopenia are found to have large mean platelet volume, lower agglutination to ristocetin and lower protein level of glycoprotein Ib-β than control patients [389]. Thrombocytopenia is found in patients with hemizygosity of chromosome 22q11.2 region [408], such patients require total management [387]. The autoimmune diseases such as juvenile rheumatoid arthritis, idiopathic thrombocytopenia, autoimmune neutropenia, grave’s disease and vitiligo are more frequent in 22q11 DS. The immune thrombocytopenic purpura is about 200 times more in patients with 22q.11.2 deletion than in the general population [409]. An increased risk of developing schizophrenia has been found in patients with 22q11 DS and thrombocytopenia [387]. Few studies have shown an increased mean platelet volume with a strong negative correlation between the mean platelet volume and platelet count in case of 22q11 DS [410].
5.1.2 AIM AND RESEARCH HYPOTHESIS

Although few studies have shown megathrombocytopenia in patients with 22q11 deletion and DiGeorge syndrome, but the association of this feature with different types of conotruncal defects is not well defined. There is no study showing association between platelet parameters and chromosome 22q11 deletion in children with conotruncal defects from India.

Specific aim of the study

The aim of this retrospective study was to evaluate mean platelet volume and platelet count in children with and without 22q11 deletion and conotruncal defects.
3.2 PATIENTS AND METHODS

In a retrospective analysis, microdeletion of chromosome 22q11 and platelet parameters were observed in 193/254 children with different lesions of conotruncal defects. Missing data in 61 cases was due unavailability of platelet parameters, since this part of study (Chapter 3) was not the primary objective. Retrospective examination of the medical records of those patients for whom peripheral blood counts and MPVs were available was done. The MPV and platelet count in 39 children with chromosome 22q11 deletion were compared with 154 cases without deletion. The details about the platelet parameter were taken from hospital medical records. The MPV and platelet count were determined in EDTA blood within 2 hours. The test was performed in an automated hematological analyzer (Abbott Cell-Dyn 3700). The MPV >10 fL and platelet count < 150 x10^9/l were considered abnormal. The genetic study included karyotyping and Fluorescence in situ hybridization (FISH) technique using TUPLE1 probe. Patients were categorized by echocardiography into seven groups as discussed in Chapter 1, section 1.2.

3.2.1 STATISTICAL METHODS

The mean platelet volume and platelet counts were compared in children with and without deletion. Continuous variables were expressed as mean and standard deviation (SD). Chi-square test was done to find the statistical significance of the difference in percentage of platelet parameters between the two groups (with and without deletion). The mean of platelet count and MPV were analyzed by independent ‘t’ test in 33/39 children, since remaining 6 patients had MPV > 20 fL. A p-value < 0.05 was assumed as statistically significant.

Besides these, to determine the best cut-off value of MPV for predicting deletion, a receiver operating characteristic (ROC) curve analysis was performed, the area under the ROC curve was calculated. From the ROC curves, the best cut-off value was determined and, at that point, sensitivity and specificity were also determined. The correlation coefficient between MPV and platelet count was also found.
3.3 RESULTS & DISCUSSION

The frequency and severity of the clinical manifestations are found to be heterogeneous in individuals with microdeletion of chromosome 22q11.2 region [12,132]. Cardiac malformations, developmental delay, dysmorphic facial features and immunodeficiency are some of the most common characteristics of 22q11 DS. In addition, haematological problems in the form of autoimmune thrombocytopenia, pancytopenia and Evan’s syndrome have also been noticed in 22q11 DS [407]. Macrothrombocytopenia has been suggested as one of the feature of wide spectrum of phenotypic features found in 22q11 deletion syndrome.

A total of 193/254 patients fulfilling the selection criteria were included in the study. The mean age was 7.27 months (SD - 7.12). The children were grouped into 2 based on deletion status, which comprised of 39 patients with deletion and 154 without deletion. Cytogenetic analysis showed normal karyotype in all patients.

3.3.1 MEAN PLATELET VOLUME IN CHILDREN WITH AND WITHOUT DELETION

It is observed that large platelets are often found to be associated with a number of haematological disorders. In the present study, the frequency of high MPV in deletion versus no deletion group was 49% and 7% (p <0.0001), which suggests that children with a 22q11 deletion have significantly high mean platelet volume as compared to those without deletion (Table 3-1).

<table>
<thead>
<tr>
<th>Status</th>
<th>Mean of MPV</th>
<th>Standard deviation</th>
<th>Standard Error Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion (n=39)</td>
<td>10.46 fL</td>
<td>2.59</td>
<td>0.125</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No deletion</td>
<td>7.58 fL</td>
<td>1.55</td>
<td>0.442</td>
<td></td>
</tr>
</tbody>
</table>
The high MPV in individuals with 22q11 deletion may be due to haplo-insufficiency of GPIbβ gene on the chromosome 22q11.2 region [387]. However, high MPV cannot be related to congenital heart defect since patients with 22q11 deletion but without cardiac defects were also found to have high MPV [410]. A comparable MPV was found between this study and other series of patients (Table 3-2) with 22q11 deletion syndrome [382,408,410].

**Table 3-2 Comparison of Mean platelet volume and platelet count in different studies**

<table>
<thead>
<tr>
<th>Publications</th>
<th>Total cases</th>
<th>MPV (fL)</th>
<th>Mean PC (x 10⁹/L)</th>
<th>Correlation between MPV &amp; PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawrence et al (2003)[391]</td>
<td>129</td>
<td>10.0</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Latger et al (2004)[404]</td>
<td>34</td>
<td>10.6</td>
<td></td>
<td>- 0.78</td>
</tr>
<tr>
<td>Naqvi et al (2011)[378]</td>
<td>20</td>
<td>10.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Our study</td>
<td>33</td>
<td>10.5</td>
<td>225 ± 80.7</td>
<td>- 0.089</td>
</tr>
</tbody>
</table>

MPV, Mean platelet volume; PC, platelet count

It was observed that the area under the receiver operating characteristic (ROC) curve for MPV in predicting deletion in individuals with conotruncal defect was 0.91 (95% CI 0.86 to 0.96) (p <0.001) (Figure 3-1). A high area indicates that MPV can be a useful predictor of 22q11 deletion in children with conotruncal defects. 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%. While, if cut off value greater than 10.0fL (international cut off value) was used as an indicator of 22q11 deletion then sensitivity of only 36% and specificity of 52% was noticed. Hence the cut- off value of 8.32 fL may be used as an indicator of 22q11 deletion in children with syndromic conotruncal defect. However, it is seen
that this marker is highly variable and dependent on a number of confounding factors, which are practically difficult to correct.

**Figure 3-1. ROC curve of MPV as a predictor of 22q11 deletion**

The Mean platelet volume can be detected in a short time by a simple blood test which is cheaper. Although, there is no important clinical inference of this haematological parameter, but the strong association between high MPV and chromosome 22q11 deletion confirms MPV as a potential useful phenotypic marker for 22q11 deletion in patients with conotruncal malformations. This would also facilitate the identification of patients to be tested for 22q11 DS. The knowledge of 22q11 deletion can guide for transfusion of cytomegalovirus negative, irradiated blood products to prevent potentially associated graft versus host reaction especially during cardiac surgery. The rationale for identifying high-risk individuals with 22q11 deletion is better management and in counseling the family.
3.3.2 PLATELET COUNT IN CHILDREN WITH AND WITHOUT DELETION

The individuals with 22q11 deletion syndrome are also found to be associated with thrombocytopenia (low platelet count) [395]. The degree of thrombocytopenia is the most important risk factor predicting bleeding. The current study shows that in deletion versus no deletion group, the mean platelet count was found to be 225 x10^9 /L (range 144 - 306 x10^9 /L) vs. 339 x10^9 /L (range 212 - 466 x10^9 /L) (p= <0.001). Thrombocytopenia was observed in 15.8% cases with deletion and 7% in those without deletion. One of the previous studies has showed thrombocytopenia in 7% of 112 individuals with deletion, it was more frequent in patients with deletion as compared to those without deletion [395]. Another study reported thrombocytopenia in 35% (13/34) of the patients with deletion but this result was not linked with bleeding tendencies even during heart surgery [408]. In the present cohort of children no significant association was found between platelet count and deletion (p=0.11).

It is noticed that patients with Bernard-Soulier syndrome are found to be heterozygotes with deletion of one copy of GpIbβ on chromosome 22q11.2 region and a mutated GATA binding site in the promoter of the remaining GP Ibβ allele [412]. The patients with BSS are found to have low platelet count, the explanation for this is loss of membrane stability which may decrease platelet survival in the circulation [413]. In individuals with 22q11 DS, a false low platelet count can be detected along with giant platelets, the reason been large platelets may be counted as leukocytes by automated cell counters [414]. Besides these, in EDTA anticoagulant platelet aggregation and clumping may occur, which can be a comparatively common cause of pseudothrombocytopenia in 15% to 20% patients with isolated thrombocytopenia [415,416]. In patients with 22q11 deletion the specific cause of thrombocytopenia is not clear [417], however in some patients, the mechanism is obviously autoimmune [407,418,419].

In individuals with 22q11 DS, immune thrombocytopenic purpura is about 200 times more common than in the general population [420,421], it is believed that this is mainly linked to immunodeficiency, a characteristic feature of
22q11 DS [420]. The present study also indicates that thrombocytopenia in patients with conotruncal defect may not be due to chromosome 22q11 deletion, more studies should be carried out to understand the etiology behind thrombocytopenia in 22q11 DS.

3.3.3 MEAN PLATELET VOLUME IN DIFFERENT SUBTYPES OF CTHD

Based on the subtypes of conotruncal defects, the frequency of high MPV varied in patients with chromosome 22q11 deletion, maximum been 100% (3/3) in case of interrupted aortic arch and none in seven patients with conoventricular ventricle septal defect. In case of patients with tetralogy of Fallot with absent pulmonary valve, neither deletion nor increased MPV was observed. The present study showed frequency of MPV to be high in children with 22q11 deletion and severe form of conotruncal defects like interrupted aortic arch, TOF/PA as compared to less severe type like tetralogy of fallot (Table 3-3).

<table>
<thead>
<tr>
<th>Deleted patients (n =39)</th>
<th>TOF n=9; 23%</th>
<th>TOF/PA n=12; 31%</th>
<th>DORV (n=4; 10%)</th>
<th>CVVSD n=7; 18%</th>
<th>TA n=4; 10%</th>
<th>IAA n=3; 8%</th>
<th>Total n=39; 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>High MPV</td>
<td>4(44%)</td>
<td>9(75%)</td>
<td>2(50%)</td>
<td>0(0%)</td>
<td>1(25%)</td>
<td>3 (100%)</td>
<td>19 (49%)</td>
</tr>
</tbody>
</table>

N, Number of cases; TOF, tetralogy of Fallot; TOF/PA, tetralogy of Fallot with pulmonary atresia; DORV, double outlet right ventricle; CVVSD, conoventricular ventricle septal defect; TA, truncus arteriosus; IAA, interrupted aortic arch.

It is presumed that more extensive study with large sample size on gene gene interaction between GPIbβ and its adjacent genes on chromosome 22q11.2 is needed, in order to understand the disparity in frequency of high MPV in deleted patients with different lesions of conotruncal defects.
3.3.4 ASSOCIATION BETWEEN MEAN PLATELET VOLUME AND PLATELET COUNT

The large platelets are functionally more reactive and are often linked with a shortened bleeding time [389]. The association between MPV and the platelets count is not clear [422]. Some of the studies have shown increased platelet size >10 fL, with a strong negative correlation between the mean platelet volume and platelet count in individuals with 22q11 deletion syndrome [387,410]. In the current study, a non significant negative correlation was found between MPV and platelet count (r = -0.089; p = 0.63) in 39 patients with 22q11 deletion (Table 3-2), on the contrary, VanGeet et al have reported a significant negative correlation (correlation coefficient - 0.583; p < 0.0001) in 38 patients with microdeletion of chromosome 22q11 [410]. Another study also showed strong negative correlation between MPV and platelet count (correlation coefficient = -0.78) [408]. In the current study, poor correlation was also found between MPV and platelet count (Correlation coefficient r = -0.292; p = < 0.0001) in patients without deletion, however the p value was found to be significant, which may be due to large sample size of 154 children.

A number of studies have described that increase in platelet volume are often associated with decreases in platelet count [423,424], possibly as a consequence of small platelets being consumed in order to maintain a constant platelet functional mass [425]. A probable cause for megathrombocytopenia is that platelet genesis depends on the GPIb/V/IX complex-membrane density and deficiency of this complex as a result of 22q11 deletion, leads to bigger and thus less platelet [408]. Normally an inverse correlation is found between MPV and platelet count [429], however in this study, it was found that only three patients with deletion had both high MPV and low platelet count, indicating that platelet count and platelet volume may be controlled by independent mechanism [426].
3.3.5 ADVANTAGES AND LIMITATIONS

The results of this study suggest that mean platelet volume above 8.3 fL can be an indicator of high risk of 22q11 deletion in patients with conotruncal defects. Patients with high MPV can easily be identified during routine haematological assay. The study confirms that MPV can be time-saving and cost effective screening marker to make a decision on when to perform the deletion testing in case of syndromic conotruncal defects. In case of unavailability of genetic diagnosis, this parameter could possibly assist high risk patients suspecting deletion for an emergency intervention. A prospective study with larger number of patients is needed to evaluate the cause of low platelet count in 22q11 deletion syndrome. The cause of thrombocytopenia may not be deletion of chromosome 22q11.2 region but some secondary factors may be responsible for it. 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.