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ORIGINAL ARTICLE

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Platelet parameters in children with chromosome 22q11 deletion and conotruncal heart defects

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Abstract

Background and Objectives: The 22q11 deletion syndrome is associated with a wide spectrum of phenotypic features, hence clinical diagnosis is difficult. Individuals with this syndrome are found to have a risk of developing transfusion associated graft-versus-host reaction, if they are given nonirradiated blood. Our aim was to elucidate whether chromosome 22q11 deletion in children with syndromic conotruncal heart defects is associated with platelet abnormality.

Materials and Methods: The genetic analysis was performed by standard cytogenetic and Fluorescence in situ hybridization technique. The platelet parameters in 39 patients with chromosome 22q11 deletion were compared with 154 cases without deletion.

Results: In deletion versus no deletion group, the mean of mean platelet volume (MPV) was 10.5 ± 2.5 vs 7.6 ± 1.5 fL, platelet count was 225 ± 80.7 and $339 \pm 127.3 \times 10^9$/L and frequency of high MPV was 49% vs 7% ($P < .0001$). The MPV was associated with a sensitivity of 90.9% and a specificity of 79.6% at a cutoff value of 8.32 fL, (area under the ROC curve 91%). A nonsignificant negative correlation was found between MPV and platelet count ($r = -0.152$; $P = .361$) in children with deletion.

Conclusion: A cutoff value of 8.32 fL for MPV can be an indicator of high risk of chromosome 22q11 deletion in individuals with syndromic conotruncal defects. Individuals with chromosome 22q11 deletion should be given irradiated blood especially during cardiac surgery. Further investigation should clarify the etiology behind variation in frequency of high MPV in different conotruncal lesions.

KEYWORDS
22q11 deletion syndrome, conotruncal heart defects, mean platelet volume, platelet volume

1 | INTRODUCTION

The 22q11 deletion syndrome (22q11 DS) is associated with a broad spectrum (more than 180) of phenotypic features,[1] including cardiac defects, developmental abnormalities, megathrombocytopenia, psychiatric disorders and learning disabilities,[2–5] hence, the clinical diagnosis of this syndrome is often difficult. The patients with 22q11 DS are found to have a risk of developing transfusion associated graft-versus-host reaction, if nonirradiated blood is given to them.[6] In developing and underdeveloped countries, genetic testing is impossible for all patients, due to expensive technique and/or unavailability of genetic facilities, hence identifying blood markers which are not costly can be useful. Although few studies have shown megathrombocytopenia in patients with 22q11 DS, but the association of platelet abnormality with different types of conotruncal heart defects is not well defined. The aim of this retrospective study was to evaluate whether chromosome 22q11 deletion is associated with platelet abnormality in Indian children with syndromic conotruncal heart defects.

2 | METHODS

In this retrospective study, 193 children with syndromic conotruncal defects underwent cytogenetic, molecular cytogenetic and biochemical evaluations. Informed parental consent was taken for all patients. The study was prospectively reviewed and approved by the Institutional ethics committee of Amrita Institute of Medical Sciences and Research Centre (AIMSRC), Kochi, India. The details regarding demographic and clinical features were retrieved from hospital medical records. The conotruncal cardiac defects were identified using standard echocardiographic techniques and categorized as follows: tetralogy of Fallot (TOF), TOF with pulmonary atresia (TOF/PA), TOF with absent pulmonary valve (TOF/APV), double outlet right ventricle (DORV), conoventricular ventricle septal defect (CVVSD), truncus arteriosus (TA), and interrupted aortic arch (IAA type A, B, C). The genetic analysis was done by standard cytogenetic (karyotyping) and fluorescence in situ hybridization (FISH) technique using TUPLE1 probe (Vysis) in 193 cases. The platelet parameters were measured at the time of recruitment of patients in an automated hematological analyzer (Abbott Cell-Dyn 3700) using EDTA blood within 2 hours of collection. The mean platelet volume (MPV) and platelet count in 39 children with chromosome 22q11 deletion were compared with 154 cases without deletion. The value of calcium was available for 23 children with high MPV. The MPV value >10 fL and platelet count < 150 × 10^9/L were considered abnormal.

Statistical analysis was performed using SPSS (version 13.0). The statistically significant difference in percentage of platelet parameters between deletion and no deletion group were observed by chi-square test. Receiver operating characteristics (ROC) curve analysis was used to estimate the best cutoff value for MPV, which distinguishes deletion and no deletion group. The mean of platelet count and MPV was analyzed by independent sample *t* test. Pearson's product—moment correlation coefficient between MPV and platelet count in two groups was also determined.

3 | RESULTS

The mean age of the total cohort of children was 7.27 months (SD = 7.12). There was no significant difference in the mean age of children with deletion 7.3 months (SD = 9.2) and individuals without deletion 7.1 months (SD = 6.38). Cytogenetic analysis showed normal karyotype in all patients. The chromosome 22q11 deletion was observed in 20% children using FISH technique. The children with deletion were found to have significantly increased platelet size *as* compared to those without deletion (Table 1). The frequency of high MPV in children with chromosome deletion was 49% as compared to 7% in those without deletion ($P < .0001$). At a cutoff value of 8.32 fL for MPV, sensitivity of 90.9% and specificity of 79.6% was noticed. The area under the ROC curve for MPV was 91% (95% CI 86%-96%) ($P < .001$) (Figure 1).

In deletion versus no deletion group, the platelet count was found to be 225 × 10^9/L (range 144–306 × 10^9/L) vs 339 × 10^9/L (range 212–466 × 10^9/L) ($P < .001$). This study showed thrombocytopenia in 15.8% cases with deletion and 7% without deletion. There was significant association between 22q11 deletion and thrombocytopenia. The correlation coefficient between MPV and platelet count in 39 patients with deletion was $r = -0.152$; $P = .361$. While, the correlation coefficient between MPV and platelet count in 154 patients without deletion was $r = -0.295$; $P \leq .0001$.

TABLE 1 Association between mean platelet volume, platelet counts and deletion status

Parameters	Mean (SD)		*P* value
	With 22q11 deletion (n = 39)	Without 22q11 deletion (n = 154)	
Mean platelet volume (fL)	10.46 (2.59)	7.58 (1.55)	< .001
Platelet count (10^9/L)	225 (81)	339 (127)	< .001

The seven types of conotruncal lesions in total cohort of 193 cases included TOF in 40% of patients, TOF/PA in 24%, TOF/APV in 3.4%, DORV and CVVSD in 13% each, TA in 4.5% and IAA in 2% of cases. The frequency of high MPV in total cohort was 6% in TOF, 19% in case of TOF with pulmonary atresia, 13% in double outlet right ventricle, 12.5% in truncus arteriosus, none in CVVSD and TOF with absent pulmonary valve and 75% in case of interrupted aortic arch, but in this type of rare lesion sample size was less, only 2% of total 193 cases. Based on the subtypes of conotruncal defects, the frequency of high MPV varied in 39 patients with chromosome 22q11 deletion, maximum been 100% (3/3) in case of interrupted aortic arch, 44% (4/9) in case of TOF, 75% (9/12) in TOF with pulmonary atresia, 50% (2/4) in double

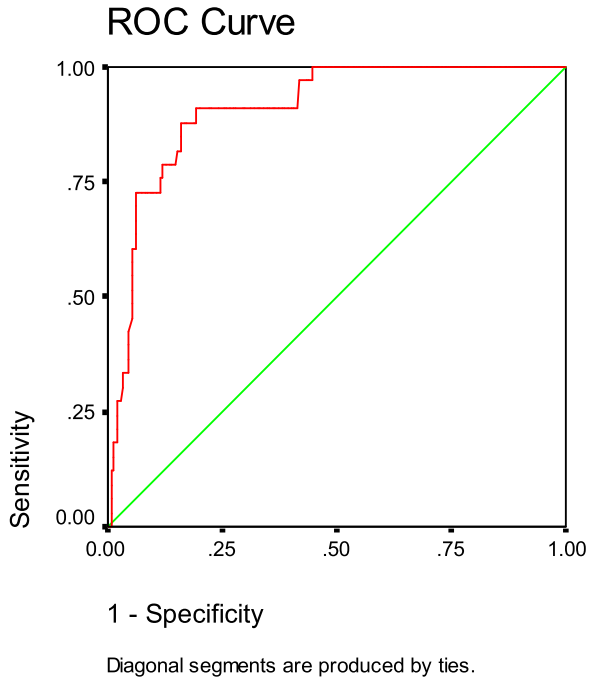

FIGURE 1 Receiver operating characteristics curve for mean platelet volume

outlet right ventricle, 25% (1/4) in case of truncus arteriosus and none in seven patients with conoventricular ventricle septal defect. In case of patients with TOF with absent pulmonary valve (n = 6), neither deletion nor increased MPV was observed.

In addition, these children also had other phenotypic features besides heart defects, the comprehensive detail of which is shown in one of our previous study.[4] The hypocalcemia was noticed 13% of total cohort of patients (n = 173). A significant difference in frequency of hypocalcemia observed in 33% (n = 39) children with chromosome deletion as compared to 7% (n = 134) in those without deletion *(P < .0001)*. The occurrence of hypocalcemia in individuals with high MPV was 35% (n = 23), while 9% in those with normal MPV (*P* = .0004). In 61% of 23 patients with high MPV, 22q11 deletion was detected. 42% of patients with 22q11 deletion exhibited normal MPV and calcium levels. Hypocalcemia with high MPV was found in 50% (8/16) cases with deletion and none in those without deletion.

4 | DISCUSSION

4.1 | 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 hematological disorders. In our cohort of children, *a* significantly high MPV was found in individuals with haploinsufficiency of 22q11 region when related with those without deletion. The etiology behind this may be haplo-insufficiency of *GPIbβ* gene on the chromosome 22q11.2, which results in bigger and thus less platelet.[7] One of the recent study indicates that hematological alterations in 22q11 DS is directly linked to genetic defects, and secondary to clinical abnormalities like immunodeficiency.[8] It is noticed that high MPV cannot be related to congenital heart defect, since, patients with 22q11 DS but without cardiac defects were also found to have high MPV.[9] In concordance with our study, Naqvi et al. and Liang et al. also reported high mean of MPV values (10.6 and 10.9 fL) in cases with 22q11 deletion when compared with those without deletion.[10,11] Although the work by Gokturk et al.,[12] showed slightly low MPV of 9.95 ± 0.46 as compared to our finding of 10.5 ± 2.5 fL. The etiology behind this may be methodology uesd and type of patients, as all patients in our study showed conotruncal cardiac defects, however, more research with large sample size is needed.

A high area under ROC curve for MPV indicates that it can be useful predictor of chromosome 22q11 deletion. Using this curve, the best cutoff value for MPV was observed as 8.32 fL (sensitivity 90.9%; specificity 79.6%). It also shows that the likelihood ratio is greater than 1 which indicates an increased probability of having 22q11 deletion in children with MPV 8.32 fL and syndromic conotruncal defects. While, if we use cutoff value greater than 10.0 fL (international cutoff value) as an indicator of 22q11 deletion then sensitivity of only 36% and specificity of 52% was noticed. Hence, lower cutoff value of 8.32 fL may be used as an indicator of high risk of 22q11 deletion in children with syndromic conotruncal defect.

Few other studies also shows that mean platelet volume, an indicator of platelet function detected by simple routine blood test, can be a useful predictor of 22q11 deletion.[10,12] However, it is seen that this marker is highly variable and dependent on a number of confounding factors, which are practically difficult to correct. Unless one can confidently achieve 100% sensitivity, the use of MPV-derived probabilities does not eliminate the requirement for genetic testing for patients with conotruncal defects. Although, there is no important clinical inference of this hematological 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 deletion. In developing and underdeveloped countries, there are limitations in the availability of genetic facilities, hence such marker can be useful. As per the knowledge of authors, this is first study on Indian children showing association between high MPV and 22q11 deletion in patients with conotruncal cardiac defects.

We have also noticed that the frequency of MPV was found to be high in children with 22q11 deletion and severe type of conotruncal defect like interrupted aortic arch, TOF with pulmonary atresia as compared to less severe form like TOF. The limitation of this study is small sample size specifically in severe type of heart defects like interrupted aortic arch which has got less frequency of occurence. Hence, statistical correlation of MPV with subtypes of cardiac malformation was not done.

The mortality was found to be high in individuals with deletion and specific subtypes of cardiac defects as compared to those without deletion.[13] This simple hematological parameter MPV can facilitate the clinicians for better follow up, since a multifaceted approach is necessary in children with hemizygosity of chromosome 22q11 region. We would like to suggest that 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.

4.2 | Platelet count in children with and without deletion

The individuals with 22q11 DS are also found to be associated with thrombocytopenia (platelet count $< 150 \times 10^9$/L). The degree of thrombocytopenia is the most important risk factor predicting bleeding. In our study, thrombocytopenia was observed in 15.8% of 39 cases with deletion *a*s compared to 7% of 112 individuals[14] and 35% of 34 cases in another study, but this result was not linked with bleeding tendencies even during heart surgery.[15]

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.[16] 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.[17] 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.[18] 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.[19,20] In patients with 22q11 deletion, the specific cause of thrombocytopenia is not clear,[21] however, in some patients, the mechanism is obviously autoimmune.[22–24] In individuals with 22q11 DS, immune thrombocytopenic purpura is about 200 times more common than general population,[11,25] it is believed that this is mainly linked to immunodeficiency, a characteristic feature of 22q11 DS.[25] Hence, we would like to indicate that mild thrombocytopenia in patients with conotruncal defect may not be due to chromosome 22q11 deletion, but some secondary factors may be responsible for it.

4.3 | Association between mean platelet volume and platelet count

The association between MPV and the platelets count is not clear in 22q11 deletion syndrome.[11] 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.[7,9] Van Geet et al. have reported a significant negative correlation (correlation coefficient = 0.583; $P < .0001$) in 38 patients with microdeletion of chromosome 22q11.[9] Another study also illustrated a strong negative correlation between MPV and platelet count (correlation coefficient = −0.78) in 34 patients.[14] In one of the recent studies, the MPV/platelet $\times 10^5$ ratios (5.36 vs 2.08, $P < .001$) were found to be higher in cohort with 22q11 DS as compared with the control group.[12] On the contrary, current cohort of children with deletion showed statistically nonsignificant negative correlation between MPV and platelet count ($r = -0.152$; $P = .361$). In addition, this study demonstrates that the correlation coefficient between MPV and platelet count in 154 patients without deletion was statistically significant ($P \leq .0001$), which may be due to large sample size, however, the magnitude of the correlation was not found to be high ($r = -0.295$).

A number of studies have described that increase in platelet volume are often associated with decreases in platelet count,[10,12] possibly as a consequence of small platelets being consumed to maintain a constant platelet functional mass.[26] 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.[15] Normally an inverse correlation is found between MPV and platelet counts.[26] Although, in the current study, it was noticed that only 7.6% patients with deletion have both high MPV and low platelet count, indicating that platelet count and platelet volume may be controlled by independent mechanism.[27]

4.4 | Other phenotypic features

In a previous study, we demonstrated that owing to 22q11 microdeletion, such children were more likely to show dysmorphic facial features (low set ears, dysplastic flared pinna, short palpebral fissures, bulbous nasal tip, microstomia, high arched palate), thin long fingers and hypocalcemia as compared to those without deletion.[4] This study showed hypocalcemia in 35% patients with increased platelet volume. Hypocalcemia with increased size of platelets were found in 50% children with deletion and none without deletion. A significant increase in frequency of hypocalcemia observed in individuals with deletion as compared to those without deletion. The occurrence of hypocalcemia in individuals with high MPV was also more when related with those showing normal MPV. The reason for high MPV and hypocalcemia in these patients may be due to deletion of chromosome 22q11.2 region.

5 | CONCLUSIONS

Our results suggest that mean platelet volume ≥ 8.3 fL can be an indicator of high risk of 22q11 deletion in patients with syndromic conotruncal defects. It indicates that chromosome 22q11 deletion is associated with platelet abnormality. It also points that in case unavailability of genetic diagnosis, MPV, a time-saving and cost effective screening marker could possibly help in better management and counseling. However, 22q11 deletion confirmation can be possible only through genetic test. The cause of high MPV and hypocalcemia in such cases may be due to chromosome 22q11 deletion, although thrombocytopenia may be due to some secondary factors. We believe that more extensive study with large sample size is needed, to understand the disparity in frequency of high MPV in patients with deletion and different conotruncal lesions.

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CONFLICT OF INTEREST

None to report.

AUTHORS CONTRIBUTIONS

Literature search and genetic testing of all patients: Anilkumar
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