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ORIGINAL ARTICLE

Prevalence of Low Platelet Count and Identification of Associating Determinants and Genetic Polymorphism in Healthy Individuals of Upper Assam, India

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Abstract The purpose of the study was to assess the prevalence of low platelet count among the healthy population of upper Assam, India. The impact of socio-demographic features was moreover pointed to evaluate. Additionally, Mean platelet volume (MPV) and Interleukin-6 gene polymorphism (-174 G > C) were also determined to speculate their effect on the basal platelet count. For determination of hematological indices, CBC was done and genetic polymorphism was identified by ARMS-PCR technique. Out of 510 study subjects, 25.3% (n = 129) had low platelet count, and females were recorded with significantly higher mean platelet count as compared to their male counterpart ($p < 0.001$). A progressive decline in platelet count was observed with ageing and more significantly noticed in females across the various age groups ($p < 0.001$). The mean MPV was significantly higher in low platelet count group as compared to the normal group ($p < 0.001$). Both platelet count and MPV differed significantly among the individuals with varied ethnicity. An inverse correlation between platelet count and its volume was reported, and such observation was continued to persist in every age-group under the study. However, no significant differences were observed for other hematological indices between the studied groups except for platelet indices and RBC count. Moreover, the peripheral blood smear examined for cellular morphology and in vitro platelet clumping did not report any significant aberrancy. No significant penetrance of the risk allele was revealed in the studied groups. However, ARMS-PCR confirmed 6% (n = 8/129) of the low platelet count subjects with heterozygous for G allele. This happens to be the first description of low platelet count among the healthy population of upper Assam, where age, gender, ethnicity, and MPV are significantly associated with platelet count variation. Heterozygosity of the risk allele does not contribute to the low platelet count condition.

Keywords Complete blood count · Ethnicity · Interleukin-6 · Platelet count · Mean platelet volume

Abbreviation

CBC	Complete blood count
ARMS-PCR	Amplification refractory mutation system-polymerase chain reaction

Introduction

Platelets are specialized blood cells responsible for maintaining the vascular integrity and hemostatic physiology in humans [1, 2]. Platelets also participate actively in numerous immune and inflammatory responses for atherosclerosis, cancer malignancies, and many hereditary disorders [3, 4]. The prime function of platelet is to arrest

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bleeding by facilitating thrombus formation at the site of injury [5, 6]. However, increased platelet activity renders unusual platelet aggregation leading to stroke and cardiovascular diseases [7]. Therefore, both quantitative and qualitative abnormalities related to platelet may cause several clinical manifestations, characteristically prolonged bleeding and/or atypical thrombus formation [8]. In a healthy individual, platelet count ranging from 1,50,000 to 4,50,000/μl of blood is considered as normal [9, 10]. However, the reference interval for platelet count varies as per the geographical location and ethnic affiliation of the population [11, 12]. Earlier studies have evidenced that Harris platelet syndrome (HPS) is the commonest inherited giant platelet disorder reported among the blood donors of the north-eastern region of India, where significantly low platelet count with raised Mean platelet volume (MPV) is observed [13, 14]. Since information related to the prevalence of low platelet count among the ethno-culturally diverse population of Assam is scanty, the present study primarily focuses on the screening of low platelet count among healthy individuals of upper Assam. Subsequent studies have also confirmed that sex and age have a significant bearing on platelet count [15]. Besides, an inverse correlation between platelet count and MPV has been reported earlier in healthy people [16]. Accordingly, the values of MPV were moreover analyzed for the study population to decipher their compensatory effect for count deficit. Other than thrombopoietin, Interleukin-6 (IL-6) also exhibits hematopoietic activity in promoting thrombopoiesis [17, 18]. It was also reported that this cytokine has a remarkable property to act as a therapeutic agent in treating thrombocytopenia [19, 20]. The molecular influence of *IL-6* gene (-174 G > C) polymorphism for platelet count variation was first described by Fernandez-real et al. in the early twenty-first century. It was reported that CC genotype associated with a significantly lower level of platelet count as compared to the carriers of G alleles in healthy individuals [21]. Since then, only a few studies have shown the role of *IL-6* gene polymorphism in platelet count variability. It is therefore important to affirm the variable genotypes as biomarkers in individuals with low platelet count.

Herein, the present study aimed to assess the prevalence of low platelet count in healthy individuals of upper Assam, India. It is also proposed to find out the possible association of factors viz.; age, gender, ethnicity, MPV, and genetic influence of *IL-6* gene (-174 G > C) polymorphism on basal platelet count.

Materials and Methods

Study Design

Based on operational and logistic feasibility, four contiguous districts of upper Assam viz.; Tinsukia, Dibrugarh, Sivasagar, and Jorhat were included in the study. A total of 510 healthy volunteers had participated in the study from both sexes (male = 46% and female = 54%) with their mean age 29.3 ± 12.6 ranging from 14 to 68 years. Signed informed consents were obtained, and social-demographic features (e.g. age, gender, locality, and ethnicity) of each individual was recorded on a pre-designed proforma. Our study reported participation of various ethnic groups representing Sino-Tibetan, Tibeto-Burman, Tai-kadai, Indo-European/Indo-Aryan, and Proto-Australoid racial group. The study protocol was approved by the Institutional Ethics Committee (IEC), ICMR-Regional Medical Research Centre, Northeast region, Dibrugarh, Assam, India from 2014 to 2016.

Subject Recruitment Criteria

Unrelated healthy subjects were recruited who had volunteered specifically for this study. Individuals with history of malaria, jaundice, thyroid disorders, anemia or any other acute infections diagnosed in the previous 6 months were excluded. Besides, individuals on medication due to hypertension, diabetes mellitus, stroke, heart attack, asthma, epilepsy, kidney diseases, arthritis or any other chronic diseases were exempted from the study. The study also excluded participants with the history of bleeding manifestation.

CBC Analysis and Microscopic Examination

Intravenous blood samples were drawn aseptically in K_3-EDTA vacutainer tubes. CBC profile of each individual was determined by Automated Hematology Analyser (Celltac α, MEK-6420K, Nihon Kohden, Japan) within 4–6 h of sample collection. Considering the reference interval for platelet count among the Indian population, a count less than 130×10^3/μl of blood was referred to as low platelet count for the present study [22]. The values of platelet count and MPV were recorded for further analysis. The peripheral blood smears of a few samples were also examined for cellular morphology and in vitro platelet clumping.

Tetra-Primer ARMS-PCR Analysis

In order to detect a single nucleotide polymorphism in *IL-6* promoter -174 G > C (NC_000007.13:g.22766645C>G/NM_000600.3:c.-116-121C>G), DNAs of the targeted blood samples were analysed employing tetra-primer amplification refractory mutation system (ARMS)-PCR [23]. The procedure utilizes the principle of tetra-primer PCR method and ARMS for detection of two different alleles in a single PCR reaction followed by gel electrophoresis [24].

Statistical Analysis

Quantitative data were presented as Mean ± (SD, Standard Deviation). Two statistical tests, namely, Independent *t* test and One-way Analysis of Variance (ANOVA) were performed to compare the mean differences between and among the study groups. For multiple comparisons, Tukey's post hoc test was conducted. The deviation level from the Hardy–Weinberg equilibrium (HWE) in the study population was calculated by an online programme (www.dr-petrek.eu/documents/HWE.xls). To compare the difference in allele distribution between the groups, Chi square test was performed, and a probability value $p < 0.05$ was considered as statistically significant. The analyses were done by using Statistical Package for Social Science (SPSS) version 17 software (SPSS Inc, Chicago, USA).

Results

Out of 510 study subjects, low platelet count was detected in 25.3% (n = 129) of the study population with mean platelet count 96.53 ± 24.79 × 10^3/µl of blood. The remaining population had normal platelet count, and the recorded mean value was 222.64 ± 71.48 × 10^3/µl of blood. A significantly higher mean platelet count ($p < 0.001$) was observed in females (207.6 ± 86.2 × 10^3/µl) as compared to their male counterpart (171.1 ± 76.1 × 10^3/µl). Age-related changes in platelet count were also observed across the various age groups. The study reported a consistent decrease in mean platelet count with ageing ($p < 0.001$), and significantly observed among females as compared to males for the same age group concern. It was also observed that the mean platelet count of participants under the age group of ≤ 20 years differed significantly from the age groups 21–30 years ($p < 0.05$), 31–40 years ($p < 0.001$) and 41–50 years ($p < 0.001$) (Table 1).

The distribution of MPV in the study population recorded a significantly raised mean value for low platelet count group (11.47 ± 1.47 fl) as compared to the normal group (10.81 ± 1.86 fl). The study also reported a consistent increase in values of MPV till the age of 50 years and then subsequently decreases. However, an inverse relationship between platelet count and size was observed and continued to sustain in every age group (Fig. 1).

It was observed that both platelet count and MPV varied significantly ($p < 0.05$) among the various ethnic groups enrolled in the study (Table 2). The major ethnic groups represented by the study population were Ahom, kachari, Mishing, Matak, and Tea Garden Workers (TGW). The miscellaneous group included Assamese Brahmin, Bengali, Muslim, Nepali, Koch, Moran, Bihari, Kaibarta, and Chutia communities. These communities were clubbed so, as their representation in the study was insufficient for individual statistical analysis. The multiple comparisons have shown that the mean platelet count of TGW was significantly different from Ahom, Kachari, and Mishing groups. Similarly, the count among Ahoms differed significantly from the Matak ethnic group ($p < 0.05$). Ethnicity-wise analysis of the data indicated that mean MPV in Kacharis differed significantly from other ethnic groups ($p < 0.05$) enrolled in the study. The test also inferred that the mean MPV in Ahoms differed from both Kachari and Matak ethnic groups ($p < 0.05$) (Table 2).

Other hematological indices were also evaluated for normal and low platelet count group. The analysis reported no significant differences between both the studied groups except for the values of platelet indices and RBC count (Table 3). The peripheral blood smears examined for a group study samples (n = 49) did not confirm any evidence for abnormal cellular morphology or/and in vitro platelet clumping. However, the presence of large size platelets was confirmed during the microscopic examination. "Online Resource 1 (Fig. 1)" "Supplementary Material".

The genotype identification of *IL-6* gene for -174 G > C polymorphism was examined for all the subjects who had low platelet count (n = 129) and in a group of individuals (n = 110) with normal platelet count. The ARMS-PCR had confirmed 6% (n = 8) of the low platelet count individuals with heterozygous (GC) genotype (Fig. 2).

The complete dominance of G allele was identified in the group of individuals with normal platelet count. The frequency of risk allele C identified in the low platelet count group was 0.03 and of allele G was 0.97. The Chi squared value of a goodness-of-fit statistics to test for Hardy–Weinberg equilibrium was 0.13 ($p = 0.71$, $df = 1$). The present study has not reported any significant penetrance of the risk allele in both the studied groups ($p > 0.05$).

Table 1 Age and gender-wise variation in platelet count across the various age groups

Platelet count, mean ± (SD) × 10^3/μl							*P* value
Age group in years	≤ 20 n = 162	21–30 n = 153	31–40 n = 98	41–50 n = 61	51–60 n = 27	≥ 61 n = 9	
Study population n = 510	219.8 ± 82.2	183.4 ± 82.2	170.4 ± 87.1	167.2 ± 72.6	188.6 ± 60.1	180.9 ± 88.8	< 0.001
Male n = 235	193.2 ± 75.9	166.3 ± 69.8	151.6 ± 79.4	162.9 ± 83.3	168.9 ± 44.0	211 ± 94	> 0.05
Female n = 275	236.3 ± 81.3	202.2 ± 90.8	186.3 ± 89.2	171.1 ± 59.6	213.2 ± 65.6	156.8 ± 65.1	< 0.001

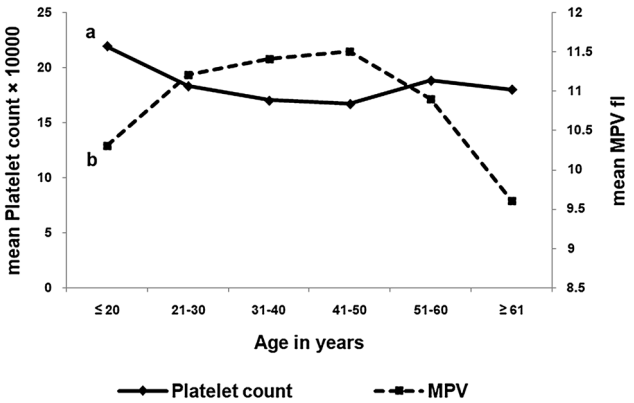

Fig. 1 Distribution of Platelet count and MPV across the various age groups. The lines representing mean values for platelet count (**a**) and MPV (**b**)

Discussion

The prevalence of low platelet count in healthy individuals is known to vary globally and thought to be distinct for the population of Assam, India [25, 26]. The inhabitants of Assam represent a heterogeneous admixture of varied ethnicity. Thus, it was assumed that platelet count could be genetically influenced and may vary among the ethno-culturally diverse population. As the inclusion of healthy individuals was the prime criterion of the study, therefore, CBC profile of each participant was important to determine. The present study has not reported any significant differences for hematological indices between normal and low platelet count group except for the values of RBC count and platelet indices. Though the mean value for RBC count was reported significantly higher in the low platelet count group of our study population, its other indices were seen to be unaffected. A similar observation was documented in a study carried out by Naina et al. [13], but the mean value for RDW was significantly raised in the donors with HPS. Therefore, it could be more informative if further investigations for the existence of Macrothrombocytopenia in this population included. Apart from that, our study had interpreted only 10% of the total sample for peripheral blood examination, and therefore, likely to be considered as one of the limitations of the study. The present study revealed 25.3% prevalence of low platelet count among the healthy population of upper Assam. Gender-wise variation in platelet count was the commonly reported figure in many studies [27] and found consistent with the findings of the present study where females had significantly higher mean platelet count as compared to male ($p < 0.001$). Studies have speculated that iron deficiency in menstruating women stimulates the production of platelets [28, 29]. However, hormonal changes in females after puberty could also play a major role in the process of thrombopoiesis [30]. Screening studies have also suggested that platelet count falls with ageing. Biino et al. [31] in their study reported a decrease in platelet count by 35% in

Table 2 Ethnicity-wise distribution of platelet count and MPV in the study population

Hematological parameters	Ethnic groups						*P* value
	Ahom n = 136	Kachari n = 120	Matak n = 52	TGW n = 51	Mishing n = 26	Miscellaneous n = 125	
Platelet count, mean ± (SD) × 10^3/μl	204.2 ± 89.9	189.8 ± 76.2	167.1 ± 89.6	156.6 ± 69.3	212.3 ± 84.1	187 ± 79.2	< 0.05
MPV in fl, mean ± (SD)	11 ± 1.8	10 ± 1.8	11.8 ± 1.5	11.4 ± 1.6	11.6 ± 1.7	11.3 ± 1.6	< 0.05

Table 3 Distribution of hematological indices between normal and low platelet count group with their mean ± SD

Hematological indices	Normal platelet count group n = 381	Low platelet count group n = 129	*P* value
Hb (g/dl)	12.6 ± 2.4	12.7 ± 2.8	> 0.05
RBC ($\times 10^6$/μl)	4.6 ± 0.8	4.8 ± 1.1	< 0.05
RDW (%)	15 ± 7.3	15.9 ± 10.2	> 0.05
MCV (fl)	76.3 ± 12.1	75 ± 10.4	> 0.05
MCH (pg)	28 ± 5.2	29.4 ± 27.3	> 0.05
MCHC (g/dl)	36.6 ± 2	35.9 ± 2	> 0.05
PLT ($\times 10^3$/μl)	222.64 ± 71.48	96.53 ± 24.79	< 0.001
MPV (fl)	10.81 ± 1.86 fl	11.47 ± 1.47	< 0.001
PDW (%)	17.2 ± 1.6	16.7 ± 1	< 0.001

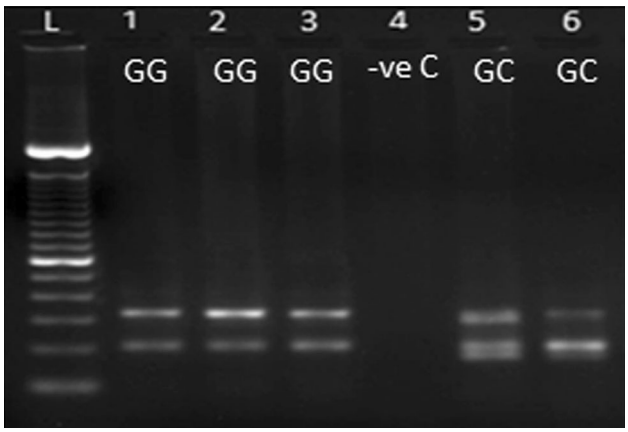

Fig. 2 Genotype identification of *IL-6* gene for -174 G > C polymorphism by tetra-primer ARMS-PCR. Results: L; 100 bp ladder, GG homozygote produces 326, 205 bp bands (*Lane* 1, 2, 3), negative control (*Lane* 4), GC heterozygote produces 326, 205, 176 bp bands (*Lane* 5, 6)

men and by 25% in women in their old age. In agreement with their findings, we also assessed significant differences in platelet count with progressive age. However, the gender-wise variation in platelet count across the various age groups was significantly observed only among females in our study population.

Platelet count varies by ethnicity and has been considered particularly to distinguish their origin [32]. The indigenous population of Assam is the assimilation of different races immigrated from countries like Tibet, Bhutan, China, Burma, and Bangladesh. Therefore, the strength of our study was participation of healthy individuals belonging to different ethnicities. Segal et al. [33] have demonstrated the ethnicity related differences for platelet count among the United States population. The more relevant information on variation for platelet count based on the ethnic origin was provided by Kueviakoe et al. [34] among the population of different African countries. The present study reported a similar observation for the ethnic impact on platelet count and MPV. Both these hematological indices varied significantly among the ethnic communities belong to Sino-Tibetan, Tibeto-burman, Tai-kadai, and Proto-Australoid racial group. There was a notable limitation regarding lower representation of individuals in some of the ethnic groups, and therefore, variations for platelet count and MPV have interpreted with caution.

In addition to many contributing factors, the present study also conferred the similar observation where a significantly high value for MPV was recorded in individuals with low platelet count as compared to the normal group ($p < 0.001$). In the absence of clinical manifestations, the presence of large size platelet in low platelet count group of individuals was most likely to be predicted for their size compensatory effect and regulated homeostatically to prevent the bleeding issues.

Further, the genome-wise studies had identified several genetic variants and markers associated with the risk of low platelet count in healthy individuals [35]. Another study conducted by Biino et al. [36] indicated the role of genetic background in regulating the platelet concentration among five different geographical isolates in Italy. Besides, works were also conducted for the genetic influence of *IL-6* gene on basal platelet count [21]. In contempt with the earlier findings, our study reported a different outcome for *IL-6* gene polymorphism with 6% prevalence of heterozygous genotype (GC) in the group of individuals having low platelet count. Based on 1000 genome—ensemble database, the frequency distribution of minor allele C was reported around 14–48% in the healthy population [37]. In the South Asian population, the analyzed frequency for C allele was reported to be 11–18% which is three–five folds higher than the observed frequency (3%) in our studied population. Therefore, considering the heterogeneity of the population of north-eastern region of India, further in-depth study is required to understand the actual frequency of the ancestral as well as the minor allele.

Conclusions

The study provides baseline information on the prevalence of low platelet count among healthy individuals of upper Assam. This happens to be the first description for illustrating the significant impact of sex, age, ethnicity, and MPV on platelet count variation. The presence of risk allele in the study population showed no significant association with low platelet count. Thus, further studies required to investigate the other genetic factors which could provide ample information for platelet count variation on the large community basis.

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Compliance with Ethical Standards

Conflict of interest The authors declares that they have no conflict of interest.

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