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ORIGINAL ARTICLE

Prevalence of Macrothrombocytopenia in Healthy College Students in Western India

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Abstract Macrothrombocytopenia is being increasingly described across the globe. There is paucity of data on the prevalence of this condition from different parts of India. 10,047 healthy college students from the city of Surat in western India were investigated for macrothrombocytopenia i.e. those with Mean platelet Volume of > 11 fL and platelet count of less than 150×10^9/L. ABO blood groups, complete blood counts, peripheral smear examination and haemoglobinopathy work up was also done. Siblings and parents of the macrothrombocytopenic individuals were also studied when available. Bleeding assessment tool of International society of thrombosis and haemostasis were applied to see if there were excessive bleeding in macrothrombocytropenia patients. One hundred and ninety-six students (1.95%) had asymptomatic macrothrombocytopenia. More female students ($P < 0.0001$) had this condition and blood group A was under represented ($P = 0.019$) with this condition. Prevalence of macrothrombocytopenia was not related to ethnic subgroups to which the students belonged to, nor was it linked to presence of any haemoglobinopathy gene. In 38 of the 52, 1st degree relatives studied macrothrombocytopenia was confirmed at least in one of them. Excessive bleeding in none of the individuals with macrothrombocytopenia was noted. Asymptomatic macrothrombocytopenia is rare in western parts of India and affects 1.95% of the healthy population. Females were over represented with this condition raising a suspicion of X linked dominant inheritance. Underrepresentation of blood group A in this condition requires further study.

Keywords Macrothrombocytopenia · Western India · Inherited · MPV-ABO blood group · Female preponderance

Introduction

Macrothrombocytopenia i.e. large platelets in peripheral blood smear may be found asymptomatically in various population in the world including India [1–3].

Several genetic polymorphisms/mutations and mono allelic forms of known inherited platelet defects which in homozygous state cause bleeding has been detected in these cases [4–6]. Though inherited and asymptomatic macrothrombocytopenia was first detected in Bengalee patient's relatives donating blood in south India [7], later on this condition was described from Northern part of India affecting 0.6% of the patients [3] who were referred for complete blood counts for different purposes. Hence prevalence data of this condition from India is not available from general population in the international/national literature. From Surat, a busy city in Gujrat state of western India we studied randomly a sizable population of healthy college students to assess the prevalence of this condition.

Materials and Methods

The study period for the present analysis is from January 2016 to November 2017.

Students attending various degree colleges in the city of Surat were evaluated. 5 ml of blood in EDTA and 2 ml

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clotted blood in vacutainer (B-D biosciences, USA) tubes were collected after counseling and taking informed consent.

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or of comparable ethical standards.

Following investigations were carried out: ABO and RhD blood group status, haemoglobinopathy, haemoglobin levels and complete blood counts through semiautomated Impedance haematology analyser 'Celltek α^{R}' (Nihon Koden, Tokyo, Japan), Leishman stained peripheral smear examination where indicated (KG & AS) were undertaken for morphology of red cells, platelets and for any abnormal inclusion in white cells. Neutrophil lobe counts were also done. The students who were shown to have macrothrombocytopenia (MPV > 11 fL with platelet count $< 150 \times 10^9$/L) were evaluated for tendency to easy bleeding by using ISTH bleeding assessment tool (ISTH-BAT) [8, 9]. Students with with anaemia (Haemoglobin < 11 g/dl for female and 12 g/dl for male) or macrocytosis (> 95 fL) or significant microcytosis (< 70 fL) and those with recent illness (defined as febrile or any illness which lasting for 7 or more days, or on any medication were excluded from the study. Haemoglobin electrophoresis in alkaline buffer and HPLC studies (Bio Rad, California, USA Variant II) along with haemoglobin solubility tests were done on all samples to screen for haemoglobinopathy, ABO and RhD blood grouping was done using standard serological techniques [10].Wherever possible siblings or parents of macrothrombocytopenic individuals were studied for mean platelet volume and platelet counts.

The study was permitted by Institutional ethics committee.

Statistical analysis for significance i.e. Student t test and Chi square test using Yates correction was done using Graphpad quickcalcs [11]. Any value of $P < 0.05$ was considered significant.

Results

During the period under study 12,059 college students (degree colleges in and around Surat city) aged between 19 and 23 years were evaluated. 2012 patients were excluded from the study as per the exclusion criteria. Finally 10,047 patients were evaluated (female 6040 and male 4007). Macrothrombocytopenia was found in total of 196 students (1.95%). Females were more often affected (150 cases) compared to that of males (46 cases) and this difference was highly significant ($P < 0.0001$, X^2 test with Yates Correction). Prevalence of macrothrombocytopenia using various cut off values for MPV were computed and presented in Table 1. Mean platelet count in all cases of macrothrombocytopenia was significantly lower compared to that of non macrothrombocytopenic students ($P < 0.0001$, unpaired t test). Peripheral blood smears of all students with macrothrombocytopenia were meticulously checked by two authors (KG & AS) to exclude any other morphological abnormality that could be associated with this condition i.e. Pelger Huet anomaly, May Hegglin anomaly, stomatocytosis, white or red cell inclusion etc. etc. There were none. There were no schistocytes which could have influenced machine platelet counts.

1/196 (0.51%) macrothrombocytopenia cases carried Sickle cell trait which was less than 302/9851 (3%) ($P = 0.0698$, X^2 Test) the study population but it was not statistically significant. We did not encounter any other haemoglobinopathy or beta thalassemia traits in the macrothrombocytopenia group though 321/9851 (3.26%) non macrothrombocytopenia patient carried Sickle cell trait as per red cell indices and HPLC data. Beta thalassemia trait was present only in 29/9851 (0.3%) population as students with significant microcytosis were excluded from the study.

None of the 196 patients had significant bleeding problem and had ISTH–BAT score of 3.1 ± 0.9. We evaluated menorrhagia separately. 16/150 (10.6%) females with macro thrombocytopenia had menorrhagia (Any of the following or combinations there of: Period lasting 5 days or more, passage of clots, 3 or more pads per day) compared to 703/5890 female students with normal platelet count and MPV less than 11 fL ($P = 0.7292$, X^2 Test with Yates correction).

Macrothrombocytopenia was specially underrepresented in blood group A population (Table 2) in the present study, however there was no significant difference of its prevalence amongst various ethnic groups of this geographical area where the study was done (Table 3). 52 first degree relatives (parents or siblings) of the macrothrombocytopenia patients could be evaluated for MPV and platelet count, 38/52 showed MPV of > 12 fl (12.2–16.7) with a mean platelet count of 133.4 (113–152) $\times 10^9$/L.

Discussion

Macrothrombocytopenia of inherited variety has been reported from diverse population groups including those from India [1–3]. However there is paucity of data on the prevalence of Inherited macrothrombocytopenia from Indian subcontinent, though individual case reports [12] and series from patients attending various hospitals or studies on their relatives have been published [3, 7]. A

Table 1 Prevalence of macrothrombocytopenia in Western India out of 10,047 healthy college students

	≥ 11 fL MPV	≥ 12 fL MPV	≥ 13 fL MPV
Total	196 (1.95%)	88 (0.88%)	45 (0.45%)
Mean ± 1SEM	11.2 ± 0.8	12.2 ± 0.7	13.1 ± 0.6
Mean ± 1SD platelet count (10^9/L)	131 ± 12.6	109 ± 15.7	101.3 ± 22.9

Platelet count in control population with (7.3 ± 0.6 fl) i.e. < 11 fL MPV and with normal platelet count (329 ± 48.6) × 10^9/L; unpaired t test $P < 0.001$ for all levels of platelet counts with higher MPV

Table 2 Major ABO blood group distribution between cases with and without macro thrombo cytopenia

	MPV ≤ 11 fL	MPV ≥ 11 fL	X^2 Test with Yates correction
Blood group A	2451 (24.88%)	34 (17.3%)	$P = 0.0194$
Blood group B	3048 (30.9%)	72 (36.7%)	$P = 0.0974$
Blood group AB	1093 (11.09%)	20 (10.2%)	$P = 0.7805$
Blood group O	3251 (33.2%)	70 (35.7%)	$P = 0.4699$

Table 3 Distribution of Macrothrombocytopenia in various ethnic subgroups compared to those with normal (< 11 MPV)

Ethnic subgroups	MPV < 11 fL	MPV > 11 fL	%
Caste population	6976 (69.43%)	125 (63.77%)	1.79
Scheduled castes/backward castes	1742 (17.33%)	43 (21.93%)	2.46
Tribes	1329 (13.22%)	28 (14.28%)	2.1
	10,047	196	1.95

None of the comparisons of above sets of data are statistically different using X^2 Test with Yates correction. ($P > 0.05$)

study from north India [3] showed 0.6% of their submitted complete blood counts showed macrothrombocytopenia (defined as MPV above 10.9 fl.) and 8 of these patients were receiving corticosteroids because of mis diagnosis of this condition with Idiopathic thrombocytopenic purpura. In that series a large number of patients with low platelet counts were actually evaluated for surgical clearence from haematology consultants. A small series of platelet apheresis donors were reported from West Bengal showing 66% of the donors have macrothrombocytopenia [13].This is a huge number and in platelet donors it poses a problem as the accepted norms for platelet count for apheresis as is written in guidelines could not be followed and this phenomenon emphasizes the need for alternative parameters like (MPV x Platelet count) to include asymptomatic macrothrombocytopenia patients as platelet donors where the prevalence of this condition is extremely high.

A large number of macrothrombocytopenia patients were evaluated for their molecular pathology at National Institute of Immunohaematology, Mumbai over last 8 years. Though more than 200 such patients were evaluated and published showing a very heterogenous molecular pathology [4–6] yet this study yielded no prevalence data as these cases were referred from all over the country. Macrothrombocytopenia cases in the present series gave no history of excessive bleeding in contrast to the study by Ali et al. which showed at least 1/3 of macrothrombocytopenia patients do have increased tendency to bleed [14]. This could have arose because of our exclusion criteria as we have excluded students with anaemia and microcytosis, who could be developing this pathology because of iron deficiency caused by chronic blood loss.

As all the students in the current study were apparently healthy we could not pick up any syndromic forms of macrothrombocytopenia from this study.

There are several interesting findings from the present data (1) significant increase in number of female patients with macrothrombocytopenia. This needs an explanation; could it be due to inherent difference in MPV between males and females? This is unlikely as there is contradictory evidence in literature either suggesting MPV in male is slightly higher [15] or there is no differences in MPV between males and females [16]. Subclinical B12 and folate deficiency or combined iron and B12/folate deficiency could cause both macroplatelets and slight diminution in platelet volume. However normal white cell count, normal neutrophil lobe counts and lack of macrocytosis in these selected individuals who has normal haemoglobin make this possibility less likely. Other rare possibilities could be the presence of large number of X linked dominant macrothrombocytopenia in the population studied [17]. Other interesting finding (2) from the present data is the significant under representation of blood group A in macrothrombocytopenia group. This finding has not

yet been reported in literature. While this could be related to rare vagaries of sampling but there could be a perfect scientific justification for the same. Blood group A transferase transfers N acetyl galactosyl moiety to suitable macromolecules (Glycoproteins and Lipoproteins) on platelet membrane and this can change the platelet membrane elasticity/stiffness in such a manner which is not conducive towards production of macroplatelets.

Increased platelet counts in umbilical cord blood samples have been correlated with blood group O [18]. One of the recent reviews [19] extensively analysed the dependence of MPV on the type of blood cell counter (i.e. Optical, Impedance etc.). It was suggested that on Advia type counters (optical) and MPV of 12.4 should be taken as the cutoff for macrothrombocytopenia and impedance based counters like the one used by the present study accentuates thrombocytopenia in macrothrombocytopenia cases and gives slightly lower MPV in these cases. Some studies have taken a cut off of 3.2 μ of Platelet diameter as evidence of macrothrombo cytopenia. Present study showed MPV of 97.5% healthy students fell between 5.6 and 10.2 fL (7.3 ± 0.6 fL, SEM). If we consider a platelet to be spherical in shape then using the formula for a sphere of 4/3 πr^3, corresponding MPV comes to 10.75 fL. Many of the modern haematology counters provide some measurement of immature platelet fraction (up to 7% could be normal). This parameter can help us to discriminate increased MPV due to large number of immature platelets seen in ITP cases from that of inherited macrothrombo cytopenia [20] One of the critic of present study could be that some of the macrothrombocytopenia cases were really cases of undiagnosed chronic ITP patients. We could not study the parents or sibs of all the students to establish inherited nature of macrothrombocytopenia in all of them however the large proportion of first degree relatives screened clearly showed the inherited nature of the condition. Chronic ITP will always come in the differential diagnosis of inherited macrothrombocytopenia. If family study is available it may sort the matter out easily, however in the absence of family study following parameter was found to be useful in one of the study [21] i.e. (1) discovery at < 34 years: positive predictive value (PPV) = 88.2%, (2) family history of thrombocytopenia: PPV = 100.0%, (3) past bleeding history of platelet type: PPV = 100%, (4) an MPV > 11 fL, (5) Many giant platelets on blood smear: 100.0%, and (6) $> 44\%$ of platelets with a surface area > 4 μm^2 in electron microscopy: PPV = 83.3% [minimum three of these positive criteria were discriminative of Inherited macrothrombocytopenia from chronic ITP with PPV = 100.0%].

This is the first study of prevalence of macrothrombocytopenia in a substantial number of randomly selected healthy population of adult students from western part of India. The present study should encourage similar studies from other parts of this country.

Authors Contribution AS & PP collected the sample analysed the results. KM collected the references and along with KG wrote the first draft of the paper. KG wrote the final paper.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest

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