

Family Screening for Thrombocytopenia in Voluntary Blood Donors with Thrombocytopenia in Kashmir Valley

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Abstract: Background: Platelets are one of the components of the blood that play an important role in clotting and bleeding. Platelets are made in the bone marrow similar to other cells in the blood such as white blood cells and red blood cells. Material and methods- This was a single centre, prospective observational study conducted in SKIMS, Soura, Srinagar, India from August 2014 to May 2016. Blood samples from voluntary blood donors, families of blood donors with thrombocytopenia, and neonates reporting to SKIMS from different areas were taken by a 18 gauge needle under all aseptic conditions and stored in an EDTA anticoagulant tube after taking proper consent from the subjects and analysed using 5 Part Differential (Sysmax XT 2000i) and peripheral blood film (PBF) examination to find out the prevalence of asymptomatic thrombocytopenia in the aforementioned population. Blood donors were screened for viral serology (HBV, HCV, HIV) and anti-nuclear antibody (ANA). Inclusion Criteria: Blood donors, families of blood donors having thrombocytopenia and neonates reporting to SKIMS, Srinagar, regardless of age and sex. Exclusion Criteria: A blood donor suffering from any disease or during convalescence of disease or has taken any agent known to cause thrombocytopenia. Voluntary blood donors with positive viral serology (HBV, HCV, HIV), or positive ANA. Results- In our study, we found thrombocytopenia in about 44.6 % of healthy blood donors of Kashmir Valley, with no identifiable acquired cause, and with absent bleeding symptoms. Majority of blood donors with thrombocytopenia have large platelets. After screening family members of voluntary blood donors having thrombocytopenia, a total of 100 families screened randomly, we found a high prevalence of familial thrombocytopenia (64.1 %) with autosomal dominant pattern of transmission and majority have large platelets. We designate this as Kashmiri macrothrombocytopenic syndrome.

Keywords: Platelets, Bone marrow

1. Introduction

Platelets are one of the components of the blood that play an important role in clotting and bleeding. Platelets are made in the bone marrow similar to other cells in the blood such as white blood cells and red blood cells. Platelets originate from megakaryocytes which are large cells found in the bone marrow. The fragments of these megakaryocytes are platelets that are released into the blood stream. The circulating platelets make up about two third of the platelets that are released from the bone marrow. The other one third is typically stored (sequestered) in the spleen [1]. Platelets, in general, have a brief 7 to 10 days lifespan in the blood, after which they are removed from the blood circulation. A normal human platelet count ranges from 150, 000 to 450, 000 platelets per microliter of blood. Thrombocytopenia is defined as a platelet count below the 2.5th lower percentile of the normal platelet count distribution. Results of the third US National Health and Nutrition Examination Survey (NHANES III) support the traditional value of 150×10^9 per L as the lower limit of normal [2]. Thrombocytopenia is defined as a platelet count of less than 150×10^3 per μL and is a common hematologic finding with variable clinical expression [3] [4] [5]. Nearly 2.5 percent of the population have a platelet count of less than 150×10^3 per μL [6]. However, platelet counts between 100 and 150×10^9 per L do not necessarily indicate disease if they have been stable for more than 6 months [2], and the adoption of a cut-off value of 100×10^9 per L may be more appropriate to identify a pathologic condition [7]. Furthermore, it is now appreciated that in many non-Western countries, like India, the lower threshold of the normal platelet count is lower

than 150×10^9 per L. Cases are considered mild if counts are between 70 and 150×10^3 per μL (70 to 150×10^9 per L) and severe if less than 20×10^3 per μL (20×10^9 per L) [6]. Patients with a platelet count greater than 50×10^3 per μL (50×10^9 per L) often are asymptomatic. Patients with a count from 30 to 50×10^3 per μL (30 to 50×10^9 per L) rarely present with purpura, although they may have excessive bleeding with trauma. However, counts from 10 to 30×10^3 per μL (10 to 30×10^9 per L) may cause bleeding with minimal trauma, and counts less than 10×10^3 per μL (10×10^9 per L) increase the risk of spontaneous bleeding, petechiae, and bruising. Spontaneous bleeding (i.e., mucosal, intracranial, gastrointestinal, and genitourinary bleeding) is more likely in patients with platelet counts less than 5×10^3 per μL (5×10^9 per L), and is considered a hematologic emergency [8].

2. Materials and Methods

This was a single centre, prospective observational study conducted in SKIMS, Soura, Srinagar, India from August 2014 to May 2016. Blood samples from voluntary blood donors, families of blood donors with thrombocytopenia and neonates reporting to SKIMS from different areas were taken by a 18 gauge needle under all aseptic conditions and stored in an EDTA anticoagulant tube after taking proper consent from the subjects and analysed using 5 Part Differential (Sysmax XT 2000i) and peripheral blood film (PBF) examination to find out the prevalence of asymptomatic thrombocytopenia in the aforementioned population. Thrombocytopenia was defined as a platelet

count of less than 150×10^9 per L on peripheral blood film examination. Blood donors were screened for viral serology (HBV, HCV, HIV) and anti-nuclear antibody (ANA).

Inclusion Criteria

Blood donors, families of blood donors having thrombocytopenia and neonates reporting to SKIMS, Srinagar, regardless of age and sex.

Exclusion Criteria

A blood donor suffering from any disease or during convalescence of disease or has taken any agent known to cause thrombocytopenia. Voluntary blood donors with positive viral serology (HBV, HCV, HIV), or positive ANA.

Statistical Analysis

In the present study, statistical package for social sciences (SPSS) v.20 was used as a software tool for analysis of data.

3. Results

A total of 1007 voluntary blood donors participated in the study, 994 (98.7%) were males and 13 (1.3%) were females. Out of 1007, 449 (44.6%) were thrombocytopenic and 558 (55.4%) nonthrombocytopenic. Out of 449 thrombocytopenics, 446 (99.3%) were males and 3 (0.7%) females. Out of 558 non thrombocytopenics 548 (98.2%) were males and 10 (1.8%) were females. .

The median age of blood donors was 30 years with a range of 18 to 60 years. Mean age of blood donors with thrombocytopenia was 30.64 ± 6.36 years (18 to 58 years) where as mean age of blood donors with normal platelet count was 30.94 ± 7.86 years (18 to 60 years). The difference in mean age was not statistically significant (p value of 0.522). Maximum number of blood donors having thrombocytopenia were seen in the age group of 20-30 years (46.3%) also maximum blood donors with normal platelet count were seen in the same age group of 20-30 years (50.5%). Mean haemoglobin of blood donors with thrombocytopenia was 13.16 ± 1.87 g/dl with a range of 8 to 18 g/dl whereas mean haemoglobin of blood donors with normal platelet count was 12.99 ± 2.07 g/dl with a range of 8 to 19 g/dl which was not statistically significant (p value=0.192). The median haemoglobin in both groups was 13g/dl. The mean corpuscular volume of blood donors with thrombocytopenia was 89.85 ± 10.11 (fL) with a range of 64.7 to 111.9 fL whereas mean corpuscular volume of blood donors with normal platelet count was 85.92 ± 34.23 fL with a range of 61 to 117.9 fL which was statistically significant with a p value of 0.019. The median MCV of blood donors with thrombocytopenia was 91 fL whereas median MCV of nonthrombocytopenic blood donors was 84 fL. The mean corpuscular haemoglobin of blood donors with thrombocytopenia was 29.42 ± 3.94 (pg) with a range of 19.9 to 41.8 pg whereas mean corpuscular haemoglobin of blood donors with normal platelet count was 27.18 ± 3.64 pg with a range of 19.7 to 39.6 pg which was statistically significant with a p value of 0.0001. The median MCH of blood donors with thrombocytopenia was 30pg whereas median MCH of nonthrombocytopenic blood donors was 28pg. The mean corpuscular haemoglobin concentration of blood donors with thrombocytopenia was 32.84 ± 4.07 (%) with a range of

24 to 39 % whereas mean corpuscular haemoglobin concentration of blood donors with normal platelet count was 32.81 ± 1.86 % with a range of 21 to 39 % which was statistically not significant with a p value of 0.893. The median MCHC of blood donors with thrombocytopenia and blood donors with normal platelet count was 33%.. Hematocrit (Hct) in blood donors with thrombocytopenia was 40.5 ± 6.29 % (25.8 to 59.9 %) compared to 39.69 ± 5.40 % (24 to 55 %) in blood donors with normal platelet count, which was statistically significant (p = 0.029). Mean platelet volume (MPV) in blood donors with thrombocytopenia was 11.97 ± 1.36 fL (8 to 15 fL) compared to 11.17 ± 1.48 fL (8 to 14 fL) in blood donors with normal platelet count, which was statistically significant (p = 0.041) or with normal platelet count, which was statistically significant (p < 0.0001). Mean platelet count of blood donors with thrombocytopenia was $101.03 \pm 27.54 \times 10^9$ per L with a median platelet count of 103×10^9 per L (23 to 149×10^9 per L). Mean platelet count of blood donors with normal platelet count was $221.59 \pm 77.46 \times 10^9$ per L (150 to 798×10^9 per L) with a median platelet count of 198×10^9 per L. Mean age of family members with thrombocytopenia was 33.16 ± 12.38 years (7 to 80 years) with a median age of 31 years where as mean age of family members with normal platelet count was 29.01 ± 11.94 years (5 to 68 years) with a median age of 26 years. The difference in mean age was statistically significant (p value of 0.001). Maximum numbers of family members with thrombocytopenia were seen in the age group of 20-30 years. (42.2%) also maximum number of family members with normal platelet count were seen in this age group only (61.3%).

A total of 100 families of voluntary blood donors having thrombocytopenia were screened for asymptomatic thrombocytopenia, 418 members of 100 families participated in study out of which 272 (65.1%) were males and 146 (34.9%) were females. Out of 418 members, 268 (64.1%) were thrombocytopenic and 150 (35.9%) were non thrombocytopenic. Out of 268 thrombocytopenics, 194 were males (72.4%) and 74 were females (27.6%). Out of 150 non thrombocytopenics, 78 were males (52%) and 72 were females (48%). Among the various red blood indices which were compared between family members with thrombocytopenia and those with normal platelets counts, MCV, MCH, hematocrit, mean platelet count were statistically significant with p values of 0.010, 0.022, 0.029, 0.0001, respectively where as MCHC, MPV and RDW were not statistically significant with p values of 0.301, 0.590 and 0.086 respectively

A total of 89 neonates screened for asymptomatic thrombocytopenia, 51 (57.3%) were males and 38 (42.7%) were females. Out of 89 neonates screened, 33 (37.1%) were thrombocytopenics and 56 (62.9%) non thrombocytopenics. Out of 33 thrombocytopenics, 18 (54.5%) were males and 15 (45.5%) were females. Out of 56 non thrombocytopenics 33 (58.9%) were males and 23 (41.07%) were females. Mean platelet count of neonates with thrombocytopenia was $97.88 \pm 25.92 \times 10^9$ per L (42 to 145×10^9 per L) with a median platelet count of 98×10^9 per L (42 to 145×10^9 per L)

L). Mean platelet count of neonates with normal platelet count was $262 \pm 124.4 \times 10^9$ per L (156 to 850×10^9 per L) with a median platelet count of 262×10^9 per L which was statistically significant (p value=0.0001). Among the various red blood cell indices compared between thrombocytopenic neonates and non thrombocytopenic neonates, Hb, MCHC, HCT, RDW were statistically significant with p values of 0.048, 0.006, 0.008, 0.014 respectively whereas MCV, MCH, MPV were not statistically significant with p values of 0.971, 0.371, 0.443 respectively.

4. Discussion

Our study reveals that asymptomatic thrombocytopenia is common in blood donors in Kashmir. This seems to be familial as 64.1% of the thrombocytopenics had more than one family member with documented thrombocytopenia. The congenital nature of thrombocytopenia was demonstrated by analysis of 89 neonates born in SKIMS ; among 51 males and 38 females, we found 33 (37.07%) to have low platelet count among which 18 were males and 15 females. The study participants were carefully questioned about any symptom of bleeding and none of the donors or their family members had a history of bleeding. Same was true of the neonates as well with no history of excessive bleeding reported by the family members or treating physicians. All the participants were screened for common known causes of thrombocytopenia like HBV, HCV, HIV, any medication use etc. None of the participants had evidence of any identifiable cause of thrombocytopenia. After ruling out the various causes of acquired thrombocytopenia like HBV, HCV, HIV, ANA, a total of 449 (44.6%) blood donors were found to have thrombocytopenia with absent bleeding symptoms with mean platelet count of $101.03 \pm 27.54 \times 10^9$ per L (23 to 149×10^9 per L) and 558 (55.4%) were non thrombocytopenic with a mean platelet count of $221.59 \pm 77.46 \times 10^9$ per L (150 to 798×10^9 per L) (p value=0.0001). After comparing various blood cell indices between blood donors with thrombocytopenia and those having normal platelet count, we found a statistically significant high mean corpuscular volume (MCV) (89.85 ± 10.11) fL, mean corpuscular haemoglobin (MCH) (29.42 ± 3.94) pg, haematocrit (Hct) (40.50 ± 6.29)%, mean platelet volume (MPV) (11.97 ± 1.36) fL, red cell distribution width (RDW) (15.99 ± 2.28)% and low platelet count ($101.03 \pm 27.54 \times 10^9$ per L) in thrombocytopenic blood donors. Majority of the blood donors with thrombocytopenia had abnormally large platelets (giant platelets), with a higher normal mean platelet volume (11.97 ± 1.36) fL. Thus kashmiris have a familial thrombocytopenic disorder with large platelets. We designate this as "Kashmiri macrothrombocytopenic Syndrome." Our data is comparable to study carried out by Harris et al [9] [10] who described a syndrome called as "asymptomatic constitutional macrothrombocytopenia" among healthy blood donors in the north-eastern part of the Indian Subcontinent characterized by absent bleeding symptoms, mild to moderate thrombocytopenia (platelet count rarely less than 50×10^3 per μ L) with a statistically high mean platelet volume (mean 10 fL), red cell distribution width and a low platelet count and platelet biomass. Giles C [11] in an analysis of 5000 unselected blood specimens showed an inverse relationship

between the number of circulating platelets and their MPV. In this study nearly 95% of normal adults the platelet count varied from 150 to 450×10^9 per L and the MPV from 7.0 to 10.5 fL. Lamparelli RD et al. [12] studied platelet counts and mean platelet volume (MPV) in 564 normal subjects using a Coulter Model S-Plus electronic counter. The mean platelet count was 283×10^9 per L while the mean MPV was 9.32 fL. A non- linear inverse correlation between platelet volume and platelet number was documented ($r = -0.38$; $p < 0.0001$). Bessman JD et al. [13] measured whole-blood mean platelet volume (MPV) and platelet count, determined by the Coulter Counter model S-Plus, in 683 normal subjects. There was nonlinear, inverse relation between MPV and platelet count throughout the normal range of platelet count: the change in MPV was most pronounced at the lower platelet counts. The mean platelet volume is dependent on a number of variables, including time of analysis after venepuncture, method of analysis, anticoagulant used and specimen storage temperature. The influence of these laboratory variables is significant and reproducible mean platelet volumes are dependent on standardized laboratory methodology. When pre-analysis factors are controlled, alterations in platelet volume can be demonstrated in a number of disease states and assessment of platelet volume can be useful in the monitoring and diagnosis of patients. An understanding of the pathophysiology of alterations in platelet volume and of the inverse relationship between platelet volume and count is a prerequisite for the successful clinical application of platelet volume measurements.[14] We did a family screening of blood donors with thrombocytopenia (a total of 100 families were screened) in order to rule out any hereditary thrombocytopenia, 418 members of 100 families were screened out of which 268 (64.1%) were thrombocytopenic and 150 (35.9%) non thrombocytopenic, reflecting a high prevalence of familial thrombocytopenia. Mean platelet count of family members having thrombocytopenia was $96.63 \pm 30.40 \times 10^9$ per litre (12 to 148×10^9 per litre) with a median platelet count of 100×10^9 per Litre. Our data suggests an autosomal dominant pattern of inheritance. The congenital nature of thrombocytopenia was demonstrated by analysis of 89 neonates born in SKIMS; among 51 males and 38 females, we found 33 (37.07%) to have low platelet count among which 18 were males and 15 females. The mean platelet count of the affected group when compared to unaffected group was 97.88 ± 25.92 vs 295.9 ± 124.36 (p value < 0.0001) On comparing various blood cell indices in neonates with thrombocytopenia and those with normal platelet count we found a statistically significant low haemoglobin (13.09 ± 4.05) gm/dl, mean corpuscular haemoglobin concentration (MCHC) (34.95 ± 2.19)%, haematocrit (37.87 ± 10.48)% platelet count ($97.88 \pm 25.92 \times 10^9$ per litre) and statistically high red cell distribution width (RDW) (18.33 ± 2.38)%. As no obvious acquired cause could be ascertained for thrombocytopenia in our study, we suggest further evaluation of thrombocytopenia including genetic studies to rule out known inherited giant platelet disorders and other congenital thrombocytopenias but congenital thrombocytopenia could not be ruled out in our study in view of strong family history and presence of thrombocytopenia at birth on neonatal screening. These subjects need to be followed for development of any subsequent systemic autoimmune

disorder that could explain the current thrombocytopenia. Genetic studies to address any underlying genetic defect as a cause of such thrombocytopenia is being planned as a part of the study and genomic wide association studies have already been initiated. We conclude that asymptomatic thrombocytopenia with autosomal dominant pattern of transmission is seen in Kashmir and the patients have large platelets. We term it as “Kashmir macrothrombocytopenic syndrome.” And we plan to exclude known causes of macrothrombocytopenia so as to elucidate the etiology of the clinical disorder.

5. Conclusion

This study was concerned with family screening for thrombocytopenia in voluntary healthy blood donors with thrombocytopenia and ascertaining the cause of such thrombocytopenia, if any. This study was carried out in outpatient setting on subjects reporting for voluntary blood donation to SKIMS, Srinagar, India from August 2014 to May 2016 after getting proper consent from the subjects. .

In our study, we found thrombocytopenia in about 44.6% of healthy blood donors of Kashmir Valley, with no identifiable acquired cause, and with absent bleeding symptoms. Majority of blood donors with thrombocytopenia have large platelets. After screening family members of voluntary blood donors having thrombocytopenia, a total of 100 families screened randomly, we found a high prevalence of familial thrombocytopenia (64.1%) with autosomal dominant pattern of transmission and majority have large platelets. Thus Kashmiris have a familial thrombocytopenic disorder with large platelets. We designate this as “Kashmir macrothrombocytopenic Syndrome.” And we plan to exclude known causes of macrothrombocytopenia so as to elucidate the etiology of the clinical disorder.

References

- [1] Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM. Wintrobe's Clinical Hematology. 10th ed. Baltimore: Williams & Wilkins; 1998. 615-83. 1557-632.
- [2] Cheng CK, Chan J, Cembrowski GS, Van Assendelft OW. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. *Lab Hematol*. 2004;10: (1):42-53.
- [3] Correia, Maria CB, Domingues, Ana LC, Heloisa R, Santos, Emilia M, Brito, Anelita et al. “Platelet function and the von Willebrand factor antigen in the hepatosplenic form of schistosomiasis mansoni”. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2009;103 (10):1053-8.
- [4] Cheung RC, McAuley RJ, Pollard JB, High mortality rate in patients with advanced liver disease independent of exposure to general anesthesia. *J Clin Anesth*. 2005;17 (3):172-6.
- [5] George JN. Platelets [review]. *Lancet*. 2000;355:1531-9.
- [6] Buckley MF, James JW, Brown DE, Whyte GS, Dean MG, Chesterman CN, et al, A novel approach to the assessment of variations in the human platelet count. *Thromb Haemost*. 2000;83 (3):480-4.

- [7] Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113 (11):2386-2393.
- [8] Cines DB, Blanchette VS. Immune thrombocytopenic purpura. *N Engl J Med*. 2002;346 (13):995-1008.
- [9] Naina HV, Nair SC, Daniel D, George B, Chandy M. “Asymptomatic constitutional macrothrombocytopenia among west Bengal blood donors”. *Am. J. Med*. 2002;112 (9):1742-3.
- [10] Naina HV, Nair SC, Harris S, Woodfield G, Rees MI. “Harris syndrome- a geographical perspective”. *J Thromb. Haemost*. 2005;3 (11):2581-2.
- [11] Giles C. The platelet count and mean platelet volume. *Br J Haematol*. 1981;48:31-37.
- [12] Lamparelli RD, Baynes RD, Atkinson P, Bezwoda WR, Mendelow BV. Platelet parameters.
- [13] Bessman JD, Williams LJ, Gilmer PR Jr. Mean platelet volume. The inverse relation of platelet size and count in normal subjects, and an artifact of other particles. *Am J Clin Pathol*. 1981;76:289-93.
- [14] Jackson SR, Carter JM. Platelet volume: laboratory measurement and clinical application. *Blood Rev*. 1993;7 (2):104-13