Evaluation of Thrombocytopenia: A Prospective Study at Sree Balaji Medical College and Hospital, India

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i20A31349
Editor(s):
(1) Dr. Jongwha Chang, University of Texas, USA.
Reviewers:
(1) Karen Cordovil, Fiocruz, Brazil.
(2) Tanya Milachich, Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Science, Bulgaria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/66751

Received 24 January 2021
Accepted 29 March 2021
Published 03 April 2021

ABSTRACT

Thrombocytopenia is a physiological deficiency in platelet counting. Fragmented RBC can be a chronic trigger for a subclinical micro angiopathy that results in chronic consumption of platelets. The platelet is a small, lentiform, anucleated cell that play a vital role in hemostasis and are produced in the bone marrow from megakaryocytes. To evaluate different etiological factors of thrombocytopenia by the study of clinical profile and laboratory parameters in patients with thrombocytopenia carried out in Sree Balaji Medical College and Hospital, Chennai. After evaluating all cases of thrombocytopenia, it is concluded that infective causes are more common than non-infective causes. Infections like dengue, malaria and septicemia were the common causes of thrombocytopenia along with megaloblastic anemia. Whenever thrombocytopenia is detected, a further investigation has to be done for specific diagnosis in the most of the cases so that appropriate treatment can be given.

Keywords: Fragmented RBC; thrombocytes; thrombocytopenia; megakaryocytes.
1. INTRODUCTION

The definition of thrombocytopenia is platelet count < 1.5 lakhs/chum. It is the commonest platelet abnormality observed in clinical practice with different clinical expression. It may result from either decreased production or increased sequestration/destruction of platelets. Destruction of platelets can be either immune or non-immune mediated. Numerous mechanisms may contribute in development of thrombocytopenia as seen in primary immune thrombocytopenia & hepatitis C virus infection. Thorough examination of the peripheral blood smear is the best way for narrowing the differential diagnosis. Megakaryocyte proliferation and platelet production are primarily regulated by interactions between thrombopoietin and its cell surface receptor, MPL [1-4]. Platelet production involves aggregation of components within the cell cytoplasm, segregation within a demarcation membrane system and organization into proplatelets [3]. The present study focuses on the evaluation of thrombocytopenia in different age groups who were established with thrombocytopenia. The platelet is a small, lentiform, anucleated cell that play a vital role in hemostasis and are produced in the bone marrow from megakaryocytes. Mature megakaryocytes extend long, branching processes, nominated proplatelets, which consists of platelet-sized swellings in tandem arrays that are connected by thin cytoplasmic bridges [4-7]. Red Blood Cells (RBC) on contact with prosthetic valves are continuously subject to damage. Fragmented RBC can be a chronic trigger for a subclinical microangiopathy that results in chronic consumption of platelets.

Megakaryocytes arise from HSCs through a common megakaryocyte–erythroid progenitor cell that gives rise to erythroid precursors and megakaryoblasts. Megakaryocytes undertake endoreduplication as they mature, emanate in large cells (30-160 um). Maturation of megakaryocyte is dependent on transcription factors GATA1 and GATA 2 together with cofactor FOG1. The nuclei of great majority of normal polyploidy megakaryocytes form irregular lobes joined by strands of chromatin.

Infectious causes dominate in tropical countries like India. In congestive splenomegaly platelet sequestration occurs by redistribution of platelets from the circulatory pool to the splenic pool. Hemodilution is seen in patients who have received colloids, crystalloids & platelet poor blood products for massive hemorrhage [8,9]. Platelets escaped identification for a long time, because of their small size and the limited resolution of early microscopes, in 1735, the German physician and poet Paul Gottlieb Werlhof provided the first detailed description of ‘morbus maculosus haemorrhagicus’ now known as immune thrombocytopenia (ITP), these blood cells were unknown [10,11]. The discovery of platelets had to wait until 1882, when the Italian pathologist Giulio Bizzozero, described in detail these small elements and the relationship between platelet adhesion and aggregation, fibrin formation and deposition [12].

One year after the brilliant insight of Bizzozero, Brohm identified the link between thrombocytopenia and ITP [6]. The intuition of Kaznelson, in 1916, that the spleen was responsible for platelet destruction led to the identification of splenectomy as a potent treatment for this disease [13,14,15]. The present study aimed to analyse the associated causes for the development of thrombocytopenia in patients admitted to Sree Balaji Medical college and Hospital, Chennai.

2. MATERIALS AND METHODS

This prospective study was conducted in the Sree Balaji medical college and hospital from march 2017 to October 2018. This study included 100 subjects who presented to the hematology department and medical OP departments of Sree Balaji Medical College and hospital. Ref. No. 002/SBMC/IHEC/2017/869.

2.1 Inclusion Criteria

Patients presenting to the hematology department and medical OP departments who were found to have thrombocytopenia, with platelet count less than 150 x 109/L in whom complete clinical and laboratory parameters were available.

2.2 Exclusion Criteria

Patients with platelet count more than 150 x 109/L, patients presented with massive hemorrhage, and who received massive colloid or crystalloid transfusion for volume loss are not included in our study. A detailed clinical history was taken. General and systemic examination was done in each patient who were included in the study population. Provisional diagnosis was made based on clinical examination and...
Peripheral venous blood was collected from antecubital vein. Appropriate amounts of blood were transferred into sodium citrate 3.2 % for estimation of coagulation profile, and tripotassium EDTA vacutainer for complete blood count. For biochemical analysis such as total leucocyte count, differential count, haemoglobin, hematocrit, Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width, platelet count, mean platelet volume and platelet distribution width, erythrocyte sedimentation rate (ESR) was measured by vesmatic cube 30 and Westergren’s method. For PT, aPTT and fibrinogen estimation, blood with appropriate amount of 3.2 % sodium citrate was used. PT, aPTT and fibrinogen was assessed by using coagulatory method in BS 390 fully automatic analyzer. D-dimer levels were also estimated in selected cases. They were stained by Leishman’s stain. For hemoparasite visualization, Giemsa stain was used. Bone marrow aspirate was taken from posterosuperior iliac crest with help of 16G bone marrow aspiration needle. Smears were stained with Leishman’s stain. Bone marrow trephine biopsy was performed in applicable cases and hematoxylin and eosin (H & E) stained paraffin sections were examined.

3. RESULTS AND DISCUSSION

In the present study maximum number of patients were in the age group 20-39 years (42 cases, ie.,42 %). (Table.1, Fig. 1).

In our study maximum number of patients presenting with thrombocytopenia were males (68%). (Table 2, Fig. 2).

The total number of our study population were analysed and categorized according to the final diagnosis. They distributed as follows. (Table .3, Fig. 3).

3.1 Diagnosis Associated with Thrombocytopenia

The most common cause observed was dengue 29% followed by malaria (15%), septicemia (13%), megaloblastic anemia (9%), liver disorder (9%), leukemia (7%), HIV (human immunodeficiency virus) infection (4%), ITP (3%) Drug induced thrombocytopenia (3%), DIC (2%), Tuberculosis (2%), Aplastic anemia (2%), MDS (1%), Hypersplenism (1%).

These cases were then analysed with the entire clinical and laboratory profiles and the possible pathogenesis outlined. (Table 4, Fig. 4).

Infective causes were found to be the most common causes of thrombocytopenia in our study.

In our study the most common cause observed was dengue 29% followed by malaria (15%), septicemia (13%), megaloblastic anemia (9%).Khatib et al. reported 10% Dengue, 29.67% malaria, 12.67% septicemia, and 15.67% megaloblastic anemia cases [16].

Kumar et al. [17] reported 32.63% of malaria, 15.78% dengue and 31.57% septicemia cases in their study [17]. Nair et al. reported 14% dengue, 9% malaria, 26% of septicemia cases in their patients with thrombocytopenia [18]. Patil et al. reported 15% dengue, 54% malaria and 4% septicemia cases [8]. Gutthi et al. reported 35% of malaria, 5% septicemia and 34% dengue cases in their study [19]. Study by paramjit et al. reported malaria 57.7%, dengue 27.7% and septicemia 4.7% cases [20]. Sanjay et al. reported dengue 30%, malaria 12.5% and megaloblastic anemia 21.6% in their study Yadav et al. reported dengue 26.8%, malaria 24.4%, and septicemia 4.4% in their study [21,22,23,24,25].

Table 1. Age distribution of cases of thrombocytopenia

| Age (years) | No. of cases | Percentage |
|-------------|--------------|------------|
| 0 – 19      | 28           | 28 %       |
| 20 - 39     | 42           | 42%        |
| 40 – 59     | 22           | 22%        |
| 60 – 79     | 8            | 8%         |
| Total       | 100          | 100%       |
Fig. 1. Age distribution of cases of thrombocytopenia

Table 2. Sex distribution of cases of thrombocytopenia

| Sex    | No. of cases | percentage |
|--------|--------------|------------|
| Male   | 68           | 68%        |
| Female | 32           | 32%        |
| Total  | 100          | 100%       |

Fig. 2. Sex Distribution of cases of thrombocytopenia
Table 3. Diagnosis associated with thrombocytopenia

| S.no | Diagnosis                  | No. of cases % |
|------|---------------------------|----------------|
| 1    | Dengue                    | 29 (29%)       |
| 2    | Malaria                   | 15 (15%)       |
| 3    | Septicemia                | 23 (13%)       |
| 4    | Megaloblastic anemia      | 9 (9%)         |
| 5    | Liver disorder            | 9 (9%)         |
| 6    | Leukemia                  | 7 (7%)         |
| 7    | HIV infection             | 4 (4%)         |
| 8    | ITP                       | 3 (3%)         |
| 9    | Drug induced              | 3 (3%)         |
| 10   | DIC                       | 2 (2%)         |
| 11   | Tuberculosis              | 2 (2%)         |
| 12   | Aplastic anemia           | 2 (2%)         |
| 13   | MDS                       | 1 (1%)         |
| 14   | Hypersplenism             | 1 (1%)         |
|      | Total                     | 100 (100%)     |

Fig. 3. Diagnosis associated with thrombocytopenia

Table 4. Pathogenesis based categorization of thrombocytopenia

| S.no | Etiological factor                              | No of cases (%) |
|------|------------------------------------------------|-----------------|
| 1    | Decreased production                           | 9 (9%)          |
| 2    | Ineffective haematopoiesis                     | 10 (10%)        |
|      | Congestive splenomegaly with                   |                 |
| 3    | hypersplenism                                  | 1 (1%)          |
| 4    | Infective causes                              | 63 (63%)        |
| 5    | Increased peripheral destruction               | 8 (8%)          |
| 6    | Liver disorders                               | 9 (9%)          |
|      | Total                                          | 100 (100%)      |
Our study included 2 cases of acute myeloid leukemia (AML) (one AML-M4) and 1 case of ALL. AML-M4 in peripheral smear showed increased number of both myeloblasts and monoblasts along with reduced number of platelets (Fig. 1) with hypercellular marrow, heterogenous cells, including immature monocytes and neutrophils. Peripheral smear of ALL showed leukoerythroblastosis, occasional reactive lymphocytes with thrombocytopenia. Bone marrow was hypercellular with infiltration by 90% lymphoblasts.

The two cases of Chronic myelogenous leukemia (CML) and one Chronic myelomonocytic leukemia (CMML) case were included in our study. Peripheral smear of CMML shows leucoerythroblastic picture with 15% myeloblasts (Fig. 2) and thrombocytopenia, bone marrow showed 43% myeloblasts, with evidence of hemophagocytosis. One case of Chronic lymphocytic leukemia (CLL) with peripheral smear showed thrombocytopenia with 63% lymphocytes, 32% neutrophils, monocytes 3%. (Fig. 3) Bone marrow biopsy showed nodular infiltration of lymphocytes. 2 cases of aplastic anaemia were included in our study, bone marrow aspiration showed hypocellular smear with a relative pancytopenia of normal hematopoietic cells along with scattered lymphoplasmacytic infiltration (Figs. 4 & 5). 9 cases of Megaloblastic anaemia were included in our study. We had one case of primary MDS in our study. Peripheral smear showed pancytopenia with leucoerythroblastic picture, macrocytes, macro - ovalocytes, nucleated RBCs with dysplasias and 4% blasts. bone marrow showed erythroid hyperplasia (70%) with dysplasia particularly in erythroid series. (Fig. 6).
Fig. 6. Photomicrograph of peripheral blood smear in a case of CMML showing myeloblasts and monocytes (Leishman 100x)

Fig. 7. Photomicrograph of peripheral blood smear showing increased number of mature lymphocytes and smudge cells background showing reduced number of platelets in a case of CLL (Leishman 100x)

Fig. 8. Photomicrograph of hypocellular bone marrow aspiration smear showing lymphoplasmacytic infiltrate and mast cells in a case of Aplastic anemia.(Leishman100x)
Fig. 9. Photomicrograph of hypocellular bone marrow biopsy showing 15% cellularity in same case of Aplastic anemia (H & E 10x)

Fig. 10. Photomicrograph of bone marrow aspiration smear showing dyserythropoiesis in a case of MDS. (Leishman 100x)

Fig. 11. Photomicrograph of peripheral blood smear showing Schizont phase of Plasmodium vivax in a patient with malaria, background shows decreased number of platelets. (Leishman 100x)
3.2 Liver Disorders

We had total 9 cases of hepatic dysfunction in our study.

CONCLUSION

Males were more commonly affected with thrombocytopenia than females in our study. Age group between 20-39 years people were more commonly affected than other age groups in our study, in most of the other studies also prevalence of thrombocytopenia is more common in this age group.

CONSENT

As per international standard or university standard, patients’ written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

ACKNOWLEDGEMENTS

The encouragement and support from Bharath University, Chennai is gratefully acknowledged. For provided the laboratory facilities to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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