RESEARCH ARTICLE

Assessment of clinical spectrum of thrombocytopenia and its association with different disease states reported in Dow Diagnostic Reference and Research Lab (DDRRL)

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Abstract

Objective: To evaluate the relevance of thrombocytopenia to other disease states in patients reporting at a haematology laboratory.

Method: The cross-sectional, retrospective study was conducted at the Dow Diagnostic Research and Reference Laboratory of the Dr Ishrat ul Ebad Khan Institute of Blood Diseases, Dow University of Health Sciences, Karachi, and comprised data from the blood bank and haematology laboratory related to patients who visited during October 2021to August 2022. Data was analysed using SPSS 26.

Results: Of the 1,249 patients with mean age 38.5±19.7 years (range: 0-89 years), 79(6.3%) were aged <12 years, 592(47.4%) were aged 12-40 years and 578(46.3%) were aged >40. Most of the patients were males 793(63.5%), and 604(48.4%) had Grade 1 thrombocytopenia. Among the females, Grade 1 thrombocytopenia was found in 246(53.9%) and Grade 2 in 140(30.7%) patients compared to 358(45.1%) and 222(28%) in males, respectively ($p<0.05$). Association between thrombocytopenia and diagnoses was not significant ($p>0.05$).

Conclusion: Thrombocytopenia was found to be strongly associated with gender.

Key Words: Thrombocytopenia, Bleeding, Dengue, Malaria.

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Introduction

The clinical significance of thrombocytopenia varies from patient to patient and is dependent on the clinical presentation. Because platelets (PLT) are necessary for maintaining vessel wall integrity, thrombocytopenia is linked to a fundamental haemostasis problem[1]. PLT count <10-20 109/L is clinically significant for spontaneous bleeding in patients. Thrombocytopenia, on the other hand, can exacerbate surgical or traumatic bleeding, as well as restrict effective treatment for a variety of illnesses[2]. In some cases, a low PLT count is the only early symptom of an underlying problem that poses a greater danger than the actual problem, or is a key indicator of disease activity, like thrombotic microangiopathies. Because of the potential repercussions on the foetus, thrombocytopenia in pregnancy requires careful attention. An organised approach to thrombocytopenia diagnosis includes integration of clinical observations as well as adequate laboratory and other medical assistance[1].

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The primary causes of a low PLT count are reduced PLT generation and increased PLT degradation. Bone marrow (BM) failure is the main reason of the former set of problems, whereas disseminated intravascular coagulation (DIC) and thrombotic microangiopathies are the reasons for the latter. PIT sequestration and haemodilution are two less familiar methods. PLT sequestration is characterised by way of the shift of PLTs from the flow pool to the splenic pool in congestive splenomegaly because of portal high blood pressure (BP)[3]. Patients with a history of haemorrhage who received colloids, crystalloids or blood, may also develop haemodilution. Some other diseases may contribute to the formation of thrombocytopenia, such as primary immunological thrombocytopenia (ITP) and hepatitis C virus (HCV) infection. Causes of thrombocytosis include major surgeries, post-splenectomy, preterm infants, haemorrhage, acute haemolysis, iron deficiency, infections, connective tissue diseases, use of cytokines, and certain drugs[4].

Thrombocytopenia can make a patient's hospital stay more difficult if they have a range of medical or surgical issues. Thrombocytopenia was discovered in about 1% of adult inpatients in an acute care hospital in one research[5]. However, only about one third of these patients manifested with bleeding symptoms. Thrombocytopenia

is substantially more common in ICUs, where it is identified in 8 percent to 68 percent of patients upon admission and 13 percent to 44 percent of patients during their stay[6]. In the acute care situation, many potential etiologies are frequently present, and it is not always easy to determine the source of the thrombocytopenia.

Even after the introduction of an advanced technology mainly at molecular and genetic level, the peripheral blood film examination remains have an important and pivotal role in driving our thrombocytopenia diagnostic strategy[7]. However, detection of thrombocytopenia in a seriously unwell patient, for the sake of diagnosis and proper treatment we want to understand whether the affected person has thrombotic microangiopathy or acute leukaemias (blasts). If effective treatment is not started soon, late or improper diagnoses may be fatal for the patient., and the reason of this mortality is that thrombocytopenia can result from a spread of pathogenic mechanisms that aren't together one-of-a-kind, and that more than on ability reasons of thrombocytopenia can be diagnosed, particularly in the extreme care setting, a simple laboratory evaluation need to encompass liver and renal feature assessments, a clotting screen with D-dimers[8] and lactate dehydrogenase measurement.

Clinical symptoms and the consequences of peripheral blood smear should elicit second-tier investigations. There may be no single haematological or biochemical test which could determine the cause of thrombocytopenia in a selected pathway[9]. A BM aspirate and biopsy should be performed to rule out a main BM problem if the cause of thrombocytopenia is unknown. Thrombocytopenia because of BM failure, which has a low chance, and destructive thrombocytopenia, which has a higher possibility, may be assessed by the use of reticulated PLTs or the similar immature PLT fraction [10,11]. Thrombocytopenia does not normally cause bleeding until the PLT count falls <50,000/L. With PLT count <20,000/L, the probability of spontaneous life-threatening bleeding increases correspondingly. To prevent spontaneous haemorrhage, most clinicians suggest PLT transfusions when levels are between 10,000/L and 20,000/L, recognising the fact that the aetiology of thrombocytopenia has obvious clinical implications[12].

The current study was planned to evaluate the frequency of thrombocytopenia, and its relevance to other disease states in patients reporting at a haematology laboratory.

Materials and Methods

The cross-sectional, retrospective study was conducted at the Dow Diagnostic Research and Reference Laboratory of the Dr Ishrat ul Ebad Khan Institute of Blood Diseases, Dow University of Health Sciences, Karachi, and comprised data from the blood bank and haematology laboratory related to patients who visited during October 2021 to August 2022. Data related to thrombocytopenia patients regardless of age and gender. Data related to healthy and disease-free individuals was excluded.

The sample size was calculated using Power Analysis and Sample Size (PASS) 15[13], based on a chi-square test with 95% confidence interval (CI), and an effect size with degree of freedom (df) 3 for the evaluation of association between thrombocytopenia grades and diseases. The power of the test for all parameters was found to >99%; dengue 0.172767, malaria 0.176348, megaloblastic anaemia 0.490377, hepatitis C (HEP C) 0.421341, and HEP B (0.568141).

Approval was obtained from the institutional ethics review board, and identity of all patients was kept confidential.

Thrombocytopenia was frequently discovered incidentally when obtaining a complete blood count (CBC) during a routine visit[14]. Based on PLT count, the cases were divided into thrombocytopenia Grades 1-4 (15); Grade 1: 75-150×10^3/µL, Grade 2: 50-75×10^3/µL, Grade 3: 25-50×10^3/µL, Grade 4: <25×10^3/µL[15].

Data was analysed using SPSS 26[16]. Categorical variables were expressed as frequencies and percentages. Association of disease with thrombocytopenia grades was checked using chi-square test. P<0.05 was considered statistically significant.

Results

Of the 1,249 patient with mean age 38.5±19.7 years (range: 0-89 years), 79(6.3%) were aged <12 years,

Table-1: Demographics and thrombocytopenia distribution.

Characteristics	Count	Percentages
Age (years)		
0 - <2	40	3.2
2 - <12	39	3.1
12 - <40	592	47.4
40 and above	578	46.3
Gender		
Female	456	36.5
Male	793	63.5
Thrombocytopenia (x10^3 /µL)		
Grade 1 (75 - 150)	604	48.4
Grade 2 (50 - 75)	362	29
Grade 3 (25 - 50)	179	14.3
Grade 4 (<25)	104	8.3

Table-2: Relationship of thrombocytopenia with gender and diseases.

Characteristics	Grade 1 Count (%)	Grade 2 Count (%)	Grade 3 Count (%)	Grade 4 Count (%)	*P*-value
Gender					
Female	246 (53.9)	140 (30.7)	47 (10.3)	23 (5)	<0.001£
Male	358 (45.1)	222 (28.0)	132 (16.6)	81 (10.2)	
Dengue					
Negative	40 (40.4)	27 (27.3)	17 (17.2)	15 (15.2)	0.097£
Positive	28 (25.5)	41 (37.3)	26 (23.6)	15 (13.6)	
Malaria					
Negative	83 (40.9)	60 (29.6)	33 (16.3)	27 (13.3)	0.113£
Positive	24 (40.7)	14 (23.7)	17 (28.8)	4 (6.8)	
Megaloblastic Anaemia					
Negative	4 (36.4)	5 (45.5)	1 (9.1)	1 (9.1)	0.131β
Positive	8 (80.0)	1 (10.0)	1 (10.0)	Nil	
HEP C					
Negative	4 (40.0)	5 (50.0)	1 (10.0)	Nil	0.292β
Positive	8 (36.4)	5 (22.7)	4 (18.2)	5 (22.7)	
HEP B					
Negative	1 (16.7)	4 (66.7)	1 (16.7)	Nil	0.113β
Positive	6 (26.1)	5 (21.7)	3 (13)	9 (39.1)	

£Chi-square Test; , βFisher Exact test, HEP: Hepatitis.

592(47.4%) were aged 12-40 years and 578(46.3%) were aged >40. Most of the patients were males 793(63.5%), and 604(48.4%) had Grade 1 thrombocytopenia (Table 1).

Among the females, Grade 1 thrombocytopenia was found in 246(53.9%) and Grade 2 in 140(30.7%) patients compared to 358(45.1%) and 222(28%) in males, respectively ($p<0.05$). Most patients were tested for malaria and dengue, but positivity for thrombocytopenia was not significant (Table 2).

Discussion

The majority of thrombocytopenia Grade 2 cases, had a high prevalence of dengue fever, those with Grade 1 carried malarial parasite as well as having low vitamin B12 and red cell folate levels, while all Grade 4 cases had either HEP B of HEP C[1,4].

The reason for thrombocytopenia in dengue fever was probably defected thrombopoiesis and the destruction of platelets in peripheral blood. The malarial parasite was probably by both non-immunological and immune mechanisms involving PLT-specific immunoglobulin G (IgG) antibodies that attach straight to malaria-associated antigen on the PLTs and causes PLT destruction whether its plasmodium (P.) vivax, P. falciparum or a mixed infection. P. vivax is the most common haematological findings with thrombocytopenia with no bleeding, no sign of anaemia or splenomegaly in most cases[17].

Thrombocytopenia is a very common complication in liver diseases, like hepatitis or cirrhosis. In the current study, Grade 4 thrombocytopenia was found in all patients having either HEP B or HEP C. Cirrhosis, splenomegaly and HEP C can be significantly found more in patients with thrombocytopenia than those having normal PLT count[18]. Usually, the spleen contains one-third of the PLTs in the body, but as the size of the spleen increases, called hypersplenism, it starts sequestering those PLTs in the peripheral blood, leaving less in circulation. However, thrombocytopenia may take place in liver cirrhosis without splenomegaly. Thrombopoietin is a hormone synthesised by liver that plays a key role in PLT production and further function[19].

The current study found that Grade 1 patients had high HEP C prevalence, for which central or peripheral auto-immune mechanism could be the cause. The hepatitis virus could start its action on thrombopoiesis by either directly invading or suppressing the BM effectiveness, which suppresses the production of megakaryocytes, or by directly affecting megakaryocytes, which reduces PLT synthesis[20]. The auto-immune destructive mechanism of mechanism of thrombocytes in HEP C is similar to other foreign invaders or auto-immune thrombocytopenic purpura, which involves attachment of virus to the receptor present on PLTs which directly charges the antibodies against those PLTs, leading to thrombocytopenia. According to a study, about 90% patients diagnosed with chronic HCV have platelet-associated immunoglobulin G (PAIgG) in their serum, and the elevated levels of PAIgG detects liver disease severity, hinting at chronic HCV having relations with the immune system[21].

One of the major findings of the current study was thrombocytopenia and its relations with vitamin B12 and red cell folate deficiency. Vitamin B12 and folate are vital cofactors in deoxyribonucleic acid (DNA) synthesis and cellular metabolism. Demethylation of homocysteine to methionine and recycling of folic acid are among their major functions. Any sort of interruption in these procedures could result in ineffective haematopoiesis and

accumulation of homocysteine. Homocysteine in excessive quantity is a toxic compound which could cause oxidative stress and inflammation in the blood vessels[22].

The current study has its limitations as it was a retrospective study based on a single-centre data.

Conclusion

Thrombocytopenia was found to be strongly associated with gender. Most patients were tested for malaria and dengue, but positivity for thrombocytopenia was not significant.

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References

1. Stasi R. How to approach thrombocytopenia. Hematology Am Soc Hematol Educ Program 2012;2012:191-7. doi: 10.1182/asheducation-2012.1.191.
2. Hayashi H, Beppu T, Shirabe K, Maehara Y, Baba H. Management of thrombocytopenia due to liver cirrhosis: a review. World J Gastroenterol 2014;20:2595-605. doi: 10.3748/wjg.v20.i10.2595.
3. Aster RH. Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. J Clin Invest 1966;45:645-57. doi: 10.1172/JCI105380.
4. Prasad V, Sharma D, Verma SL. Peripheral Blood Smear Examination: An Overview. Webology 2022;19:3170-81.
5. Teo CP, Kueh YK. Incidence of thrombocytopenia in an acute care hospital. Ann Acad Med Singap 1989;18:379-81.
6. Hui P, Cook DJ, Lim W, Fraser GA, Arnold DM. The frequency and clinical significance of thrombocytopenia complicating critical illness: a systematic review. Chest 2011;139:271-8. doi: 10.1378/chest.10-2243.
7. Palma-Barqueros V, Revilla N, Sánchez A, Zamora Cánovas A, Rodriguez-Alén A, Marín-Quílez A, et al. Inherited Platelet Disorders: An Updated Overview. Int J Mol Sci 2021;22:4521. doi: 10.3390/ijms22094521.
8. Lorenzo-Villalba N, Zulfiqar AA, Auburtin M, Schuhmacher MH, Meyer A, Maouche Y, et al. Thrombocytopenia in the Course of COVID-19 Infection. Eur J Case Rep Intern Med 2020;7:e001702. doi: 10.12890/2020_001702.
9. Israels SJ, Kahr WH, Blanchette VS, Luban NL, Rivard GE, Rand ML. Platelet disorders in children: A diagnostic approach. Pediatr Blood Cancer 2011;56:975-83. doi: 10.1002/pbc.22988.
10. Monteagudo M, Amengual MJ, Muñoz L, Soler JA, Roig I, Tolosa C. Reticulated platelets as a screening test to identify thrombocytopenia aetiology. QJM 2008;101:549-55. doi: 10.1093/qjmed/hcn047.
11. Kurata Y, Hayashi S, Kiyoi T, Kosugi S, Kashiwagi H, Honda S, et al. Diagnostic value of tests for reticulated platelets, plasma glycocalicin, and thrombopoietin levels for discriminating between hyperdestructive and hypoplastic thrombocytopenia. Am J Clin Pathol 2001;115:656-64. doi: 10.1309/RAW2-0LQW-8YTX-941V.
12. Murugan RJ. A Study on the Clinical Profile, Diagnostic work up and Follow up of Children Aged 2 Months to 12 Years with Thrombocytopenia. [Online] 2013 [Cited 2023 October 22]. Available from URL: https://core.ac.uk/download/pdf/235666091.pdf
13. Bujang MA, Adnan TH. Requirements for Minimum Sample Size for Sensitivity and Specificity Analysis. J Clin Diagn Res 2016;10:YE01-6. doi: 10.7860/JCDR/2016/18129.8744.
14. Gauer RL, Braun MM. Thrombocytopenia. Am Fam Physician 2012;85:612-22.
15. Paramjit E, Rao R, Sudhamani S, Roplekar P, Shaffi Z, Roy S. Spectrum of thrombocytopenia: A clinicopathological study with review of the literature. Muller J Med Sci Res 2016;7:121-4. Doi: 10.4103/0975-9727.185012.
16. Bestall S, Flynn R, Charleson G, Abbott PV. Assessment of Australian Dentists' Treatment Planning Decisions Based on Diagnosis. J Endod 2020;46:483-9. doi: 10.1016/j.joen.2020.01.004.
17. Gandhi AA, Akholkar PJ. Clinical and laboratory evaluation of patients with febrile thrombocytopenia. Natl J Med Res 2015;5:43-6.
18. Rios R, Sangro B, Herrero I, Quiroga J, Prieto J. The role of thrombopoietin in the thrombocytopenia of patients with liver cirrhosis. Am J Gastroenterol 2005;100:1311-6. doi: 10.1111/j.1572-0241.2005.41543.x.
19. Hancox SH, Smith BC. Liver disease as a cause of thrombocytopenia. QJM 2013;106:425-31. doi: 10.1093/qjmed/hcs239.
20. Jiang H, Li Y, Sheng Q, Dou X. Relationship between Hepatitis B virus infection and platelet production and dysfunction. Platelets 2022;33:212-8. doi: 10.1080/09537104.2021.2002836.
21. Olariu M, Olariu C, Olteanu D. Thrombocytopenia in chronic hepatitis C. J Gastrointestin Liver Dis 2010;19:381-5.
22. Lee KT, Teoh CS, Chew TK, Goh AS. Microangiopathic haemolytic anaemia and thrombocytopenia due to combined vitamin B12 and folate deficiency masquerading as thrombotic thrombocytopenic purpura. J R Coll Physicians Edinb 2020;50:144-7. doi: 10.4997/JRCPE.2020.213.