Role of Platelet Indices and Antiplatelet Antibody in Differentiating Immune Thrombocytopenic Purpura from Other Causes of Thrombocytopenia

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ABSTRACT

Background: thrombocytopenia may be defined as a decrease in number of platelets in the circulating blood. Bone marrow examination may be required to discriminate causes of thrombocytopenia as hypoproductive or hyperdestructive. However, this procedure is invasive and time consuming. **Objective:** this study aims to assess the diagnostic value of mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell-ratio (P-LCR) and anti-platelet antibody in discriminating causes of thrombocytopenia as hypoproductive or hyperdestructive immune thrombocytopenia purpura (ITP).
Patients and Methods: the study was conducted on 45 subjects15 patients suffering from ITP, 15 patients suffering from other causes of thrombocytopenia, and 15 healthy controls. All of them were subjected to full clinical history, examination and routine investigation including CBC, routine chemical analysis (liver function, renal function, etc.), BM aspirate and detection of antiplatelet antibody. **Results:** the results of this study revealed that in ITP patient group, we found that the MPV, PDW and P-LCR were significantly higher in comparison to non-ITP group. As regard anti-platelet antibody detection by ELISA method was positive (60%) in ITP group, positive (26.7) in non-ITP group and negative in all patients in control group. It revealed no significant difference between ITP group and non-ITP group to diagnose ITP. **Conclusion:** from this study, we could conclude that, measuring of platelet indices (MPV, PDW and LCR) provides useful diagnostic test in differentiating ITP from hypoproductive thrombocytopenia, thus may avoid the need for bone marrow examination. Measurement of anti-platelet antibody is not a reliable test in diagnosis of ITP.
Keywords: Immune thrombocytopenia purpura (hyperdestructive thrombocytopenia), Hypoproductive thrombocytopenia, Platelet indices (Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet Large Cell-Ratio (P-LCR) and anti-platelet antibody.

INTRODUCTION

Platelets (thrombocytes) are colorless blood cell that play an important role in processes such as hemostasis, wound healing, angiogenesis, inflammation, and innate immunity. Platelets are formed from the cytoplasm of megakaryocytes (MKs), their precursor cells, which reside in the bone marrow [1]. Platelet disorders can be either quantitative or qualitative. Qualitative disorders are due to defective platelet functions (thrombasthenia). Quantitative disorders are either due to decreased platelet count (thrombocytopenia) or increased platelet count (thrombocytosis) [2].

Thrombocytopenia may be defined as a subnormal number of platelets in the circulating blood. Normal platelet counts in adult (150–410 x 10^9/L) while in children (7-12 years old) is (170-450 x 10^9/L) [3].

The two main causes of thrombocytopenia excluding pseudothrombocytopenia are increase destruction or peripheral consumption (hyperdestructive thrombocytopenia), such as immune thrombocytopenic purpura (ITP), disseminated decreased platelet productions (hypo-production thrombocytopenia) are associated with a number of bone marrow diseases [4].

Platelet indices (PI), mean platelet volume (MPV) and platelet distribution width (PDW), platelet large cell ratio (P-LCR) are a group of derived platelet parameters obtained as a part of the automatic complete blood count. Emerging evidence suggests that PIs may have diagnostic and prognostic value in certain diseases [5]. The combined interpretation of platelet parameters is highly useful in differential diagnosis of platelet related disorders [6].

Platelet antibodies can be autoimmune (directed against endogenous, i.e., the patient's own platelet antigens) or alloimmune (directed against antigens on exogenous platelets encountered through pregnancy or transfusion). Platelet antibodies may be directed to a number of antigenic "targets" carried on platelet cytoplasmic membranes [7]. This platelet antibody

profile is designed to detect antibodies to HLA class I and platelet glycoprotein IV (CD36) antigens, and to polymorphic epitopes on the platelet GPs IIb/IIIa, Ib/IX, and Ia/IIa [8].

SUBJECTS AND METHODS
Subjects: This study was approved by the Ethics Board of Al-Azhar University.The patients were selected from Al-Azhar University Hospitals over a period from 8th June 2018 to 5th September 2018. **The study was approved by the Ethics Board of Al-Azhar University.**

They were classified according to etiology of thrombocytopenia in two groups:
Group I: Destructive Thrombocytopenia (ITP):
This group included fifteen patients with ITP. All of them were newly diagnosed ITP patients with platelet count <100.000\mm^3.
Group II: Hypoproductive Thrombocytopenia:
This group included five patients with chronic lymphocytic leukemia, with platelet count < 150.000\mm^3, they were 2 males and 3 females, with age range from 47 - 52 years, four patients with idiopathic aplastic anemia (They were 2 males and 2 females, with age range from 24 - 50 years). Three patients with myelodysplastic syndrome (they were two males and one female with age range from 40 – 48 years) and finally three patients under chemotherapy (2 breast cancer cases and using endoxan chemotherapy,1 lung cancer using gemzar chemothearpy) (they were 3 females, with age range from 49 -51 years).

Control group:
It included fifteen apparently healthy individuals with matched age and sex. They were 9 males and 6 females, with age range from 11 to 50 years.

SAMPLES AND METHODS
Full history and clinical examination.
- four milliliters (4 ml) venous blood were collected under complete aseptic conditions, each blood sample was distributed into tubes as follows:
-Two ml of blood were delivered into plastic tubes containing 1 .5 ± 0.25 mg dipotassium EDTA per 1 ml blood for performing complete blood picture on Sysmex xp 300
-Two ml of ml blood were delivered into dry plain plastic tube that were centrifuged for collection serum.

The following tests were done for both patients and control groups. They included:

1-Complete blood picture including total leucocytic count, hemoglobin, platelet count, mean platelet volume (MPV) and platelet distribution width (PDW) done by Sysmex xp 300 with microscopic examination of peripheral blood film stained with Leishman stain.
2-Detection of anti-platelet antibody detection by ELISA method.

Statistical analysis
- Data were analyzed using Statistical Program for Social Science (SPSS) version 15.0.
- Descriptive statistics used for quantitative data were; Mean ±SD while categorized data were represented as numbers and percentages.
  The following test was done: Chi-square test: was used when comparing between qualitative data.
- Friedman One way analysis of variance (ANOVA) was used to compare means of parametric data of different groups.
- Post Hoc test: Fisher's least significant difference (LSD) was used for multiple comparisons between different variables.
- For all analysis, a two-tailed test was used and p <0.05 was considered statistically significant.

RESULTS
As regard MPV results show statistically significant difference **(P1 = 0.01)** between ITP and non-ITP groups, also show statistically significant difference **(P2 = 0.004)** between non-ITP and control groups, and show non-statistical significant difference (P3 = 0.7) between ITP and control groups table (1).

As regard PDW results show statistically significant difference (P1 = 0.003) between ITP and non-ITP groups, also show highly statistical significant difference (P2 < 0.001) between non-ITP and control groups and show highly statistical significant difference (P3 < 0.001) between ITP and control groups (Table 1).

As regard P-LCR results show highly statistical significant difference (P1 = 0.001) between ITP and non-ITP groups, also show highly statistical significant difference (P2 < 0.001) between non-ITP and control groups and show highly statistical significant difference (P3 < 0.001) between ITP and control groups table (1).

As regard antiplatelet antibody results show non-statistical significant difference (P1 = 0.07) between ITP and non-ITP groups, also show statistically significant difference (P2 = 0.03) between non-ITP and control groups and show highly statistical significant difference (P3 < 0.001) between ITP and control groups (Table 2).

Table (1): comparison between studied groups as regard platelet indices

Variables		ITP (N = 15)	Non – ITP (N = 15)	Control (N = 15)	P-value
MPV	Mean	10.1	9.1	10.2	P0 = 0.008 P1 = 0.01 P2 = 0.004 P3 = 0.7
	±SD	1.4	0.9	0.4	
PDW	Mean	17.4	16.5	15.3	P0 < 0.001 P1 = 0.003 P2 < 0.001 P3 < 0.001
	±SD	1.1	0.7	0.6	
P-LCR	Mean	42.1	27.5	36.7	P0 < 0.001 P1 < 0.001 P2 < 0.001 P3< 0.001
	±SD	4.1	5.02	2.2	

- P0: statistical significance between all studied groups.
- P1: statistical significance between ITP and non-ITP groups.
- P2: statistical significance between non-ITP and control groups.
- P3: statistical significance between ITP and Control.

Table (2): comparison between studied groups as regard antiplatelet antibody

Variables		ITP (N = 15)	Non – ITP (N = 15)	Control (N = 15)	P-value
Anti-PLT Ab	Negative	6 (40%)	11 (73.3%)	15 (100%)	P0 = 0.001 P1 = 0.07 P2 = 0.03 P3 < 0.001
	Positive	9 (60%)	4 (26.7%)	0 (0%)	

DISCUSSION

Thrombocytopenia is a common clinical problem found in laboratory results during health examinations, and potentially one of the most life-threatening diseases [9]. Thrombocytopenia is often divided into two major causes' hyperdestructive thrombocytopenia and hypoproductive thrombocytopenia [10].

NO simple diagnostic test is available to diagnose thrombocytopenia pathogenesis. Bone marrow examination may be required to discriminate causes of thrombocytopenia as hypoproductive or hyperdestructive. However, this procedure is invasive and time consuming [11]. The aim of this study is to evaluate the significance of platelet indices and anti-platelet antibody in the diagnosis of thrombocytopenia by comparing the levels in hyperdestructive thrombocytopenia (ITP) and hypoproductive thrombocytopenia. MPV values of 9.1 fl for the non-ITP group and 10.1 fl for ITP patients observed in our study. In the group of ITP of this study MPV was significantly higher than non-ITP group (p=0.01).

Similarly **Kaito et al.** [12] revealed that MPV is higher in ITP than aplastic anemia which reflects increase in the production rate.

Moreover, previous work done by **Ntaios et al.** [13] reported that MPV may be safely relied on for a positive diagnosis of ITP with no false-positive or false-negative, results for diagnosis of ITP with 100% specificity, sensitivity, PPV and NPV. Likely **Shah et al.** [14] found that MPV shows higher levels in ITP patient than those in acute myeloid leukemia in which mean of MPV in ITP was 10/4+2. 03 fl in comparison to 8. 9+fl in acute myeloid leukemia.

We observed in our study higher values than those reported from **Bowles et al.** [15] and from **Chandra et al.** [16] In all these studies, they showed that MPV was significantly different between hypoproductive and hyperdestructive patients.

However, the reported mean MPV values were 8.1 fl and 9.8 fl in the Bowles study, and 7.3 fl and

8.62 fl in the Chandra study in hypoproductive and hyperdestructive patients, respectively.

This deviation in the results of the cut off value of MPV in different studies could be attributed to the difference in the selection of the patients with hypoproductive thrombocytopenia group which is reflected in the mean value of MPV in this group of patients which was 10.2±0.26 fl in the study of **Kaito et al.**[12] compared to 9.1±0.9 in our study..

Another explanation for the deviation of results of the cut off value of MPV in different studies could be the difference in the type of the hematology analyzer used as older automated analyzers which could have been used in these studies cannot discriminate platelets from other similar-sized. Particles such as fragmented red or white cells debris and immune complexes. Moreover they didn't not count large or giant platelets because they cannot be differentiated from red cells. Furthermore, many papers in the literature have shown that MPV is dependent on a number of variables including time of analysis after venipuncture, anticoagulant used, specimen storage temperature and counter technologies [17].

As for platelet-LCR, it showed 27.5±5.02 fl for the non-ITP group and 42.1±4.1 fl for ITP patients observed in our study. It was significantly higher in the group of ITP than all studied patient groups and controls (p<0.001). Nearly similar to study performed by **Ntaios et al.** [13] to diagnose ITP. Also, in the study performed by **Kaito et al.**[12] P-LCR was significantly higher in ITP than in aplastic anemia therefore it was effective in distinguishing these two types of thrombocytopenia hyperdestructive thrombocytopenia from hypoproductive thrombocytopenia.

Regarding PDW; values showed 16.5±0.7 fl for the non-ITP group and 17.4±1.1 fl for ITP patients observed in our study. It revealed significant difference between both patients' groups in our study (p=0.003), similar to study performed by **Shah et al.**[14] which found that PDW showed higher levels in ITP patients with a mean value of 18. 1±1. 9% in relation to acute myeloid leukemia patients with a mean value of 12. 1±2 1%. **Kaito et al.** [12] **also** suggested PDW has role to distinguish ITP from hypoproductive thrombocytopenia. Similarly, **Ntaios et al.** [13] suggested a cut off value of 15-17 fl with 100% sensitivity and specificity.

Similar results were obtained by **Xu et al.** [18] when comparing PDW in ITP patients and patients with BMF; they concluded that PDW of≥17 5% has a positive predictive value of 83. 9% for BMF and PWD of <16.0 % has the highest negative predictive value (58.9%) for BMF.

All platelet indices were significantly higher in ITP than in hypoproductive thrombocytopenia; thus our study concludes that increased MPV, P-LCR and PDW may provide a reliable positive diagnosis of ITP in case of thrombocytopenic patient.

As regard anti platelet antibody detection by ELISA method was positive (60%) in ITP group, positive (26.7%) in non-ITP group and negative in all subjects in control group. It revealed no significant difference between ITP group and non ITP group to diagnose ITP.

Although anti platelet auto antibodies appear to "play "a central role in the pathogenesis of ITP, some patients have no detectable antibodies at the time of diagnosis [19].

Similar to study performed by **Hamidpour et al.**[20] by ELISA method, which found anti-platelet antibodies were positive in 63.5% in ITP patients. Also studies done by **Sanjo et al .**[21] have reported that serum PAIgG levels are elevated in thrombocytopenic patients with cirrhosis with specificity for GPIIb/IIIa or ib / Ix and PAIgG was implicated by an immune mechanism in patient with liver disease because PAIgG binds to platelets, thus promoting sequestration in the reticuloendothelial system.

Also in study performed by **Al-Trabolsi** [22] by flow cytometer they found anti-platelet antibodies were positive in AITP and CITP for 80 % and 63 % of cases respectively, while in non ITP it was positive for 40 % of cases only.

In study performed by **Agarwal et al.**[23] by a combination of platelet immunofluorescence test (PIFT), it was found that platelet antibodies were positive in patients (53.7%) with leukemia, (48.3%) of the patients with aplastic anemia and (50%) of the patients with myelodysplastic syndrome .

However the study done by **Huh et al.** [24] confirmed that the measurement of PAIgG / PAIgM by flow cytometry is sensitive (74.6 %) and specific (79.7) for the diagnosis of patients.Finally, anti-platelet antibody cannot discriminate ITP from hypoproductive thrombocytopenia.

CONCLUSION

Thus, our study concludes that increased MPV, PDW and P-LCR may provide a reliable positive diagnosis of ITP in case of thrombocytopenic patients. This study points to the importance of evaluating platelet indices especially MPV, PDW and P- LCR to the thrombocytopenia investigations as they provide a reliable positive useful diagnostic test in differentiating ITP from hypoproductive thrombocytopenia, thus may avoid the need for bone marrow examination.

Measurement of anti-platelet antibody is not a reliable test in diagnosis of ITP.

RECOMMENDATION

In view of the present study, we recommended the following:

Further studies on wider scale of patients should be done. Platelet indices are recommended to be taken in consideration, as they are provided within the complete blood picture, when differentiating ITP from other causes of thrombocytopenia.

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