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Original Research Article

Clinicopathological and flowcytometric analysis of platelet function in patients presenting with thrombocytopenia

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ABSTRACT

Background: Platelet function disorders are highly heterogeneous; those may be inherited/ acquired. Patients with these may have inadequate platelet count with impaired function, but may also have both. Early detection of platelet dysfunction also helps in better patient management and improve outcome.
Material and Methods: Prospective study done in 50 cases, Patient's details history of thrombocytopenia. Complete blood count with peripheral smear is done and various parameters are analysed with respect to the platelet count, PDW, P-LCR. On EDTA preserved blood sample four monoclonal antibodies CD41, CD42b, CD61 and CD62p are applied and analysed by flow cytometer.
Results: In 50 thrombocytopenia cases out of which 06(12%) were immunological thrombocytopenia & 44 (88%) were non-immunological thrombocytopenia.

1. Introduction

Platelets are important for blood flow preservation. Whenever vascular damage occurs, platelets have role in prevention of excessive blood loss through formation of a stable plug at the site of injured vessel wall. Platelet Function Disorders (PFDs) are mostly present with spontaneous mucocutaneous bleeding, increased bleeding time or easy bruising and menorrhagia.[1]

Testing can be done via whole blood, PRP or washed platelets. Whole blood and PRP requires small sample volumes and so is helpful for the neonate.[2]

Flow cytometry is used in such conditions because- Platelet activation is minimised as sample manipulation is minimal. Multiple platelet receptors can be analysed simultaneously. Small volumes of blood are required so it can be used in patients with severe thrombocytopenia.[3]

Haemostatic functions are performed by Platelet receptors and these receptors either activate platelets.[4] Platelets are unable to perform their functions in the absence of their receptors.[5] Their work as adhesion molecules to interact with the damaged endothelium or other platelets and leukocytes. GPIb-IX-V complex is a membrane receptor complex on surface of the platelets and Cluster of differentiation 41 (glycoprotein IIb); 4 molecular weight 140 kilo Dalton [kDa]) is a glycoprotein present on platelets, megakaryocytes that mediates the platelet adhesion, facilitating binding to vWF on damaged sub endothelium, under high fluid shear stress conditions.[6–8]

CD42b reacts with GpIb present on megakaryocytes and platelets. CD42b also inhibits ristocetin-dependent binding of Von Willebrand Factor to platelets and ristocetin-induced platelet agglutination. CD61 (integrin family member) that is GPIIIa having molecular weight 110 kDa.

These glycoproteins functions as receptors for fibrinogen, von Willebrand factor (vWf), fibronectin

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and vitronectin.[9] Aggregation begins after activation of the complex of GPIIb/IIIa (CD41/CD61) receptor which allows these receptors to bind with vWF or fibrinogen.[10]

CD62p that is P-selectin / granule membrane protein 140 which is a glycoprotein of 140 kDa in the alpha granules of platelets, this is trans-located to the plasma membrane upon activation.[11]

CD62p Expression on the platelets surface membrane is associated with diabetes mellitus, acute cerebrovascular accident, acute coronary disease, peripheral vascular diseases and pre-eclampsia patients.[12]

The platelet functions most accurately can be assessed by flowcytometry such as activation, aggregation or platelet leukocyte interaction. This study was planned to evaluate platelet dysfunction in patients presenting with thrombocytopenia.[13]

2. Materials and Methods

The present study was conducted in the department of pathology M.Y. Hospital, Indore from February 2020 to June 2021. The material for the study is comprised of total 50 blood samples were taken from the patients attending outpatients and inpatient department of medicine, orthopaedics, ophthalmology with symptoms having cutaneous bleeding, rashes, Bleeding gum, redness and non -healing corneal ulcer in eyes. These patients were further sent to the department of pathology for flow-cytometric analysis of platelets.

2.1. Inclusion criteria

1. Both gender and any age.
2. Patients presenting with thrombocytopenia. (Platelet count< 1 lakh).
3. Patient confirmed by clinical evaluation suggesting thrombocytopenia.

2.2. Exclusion criteria

1. Patients with any other bleeding disorders.
2. Patients with clotting factor deficiency.
3. Patients with any other systemic disease and malignant condition.

2.3. Specimen

In this study patient's informed consent will be taken. After that proper clinical examination, detailed history will be obtained and blood sample will be received from the patients. The blood sample should be taken in an EDTA containing vial under aseptic precaution, and as soon as possible specimens are stored at 2-8ºC. The sample must be homogenized by gentle agitation, prior taking the test. The sample must be analysed within a period of 24 hours of venepuncture. This sample is used for Flowcytometric study.

2.4. Principle

The flow cytometer's basic principle is measurement of light diffusion fluorescence of the cells, used for analysis. It can select the desired population of the cells within the electronic window, showing on a histogram. The flow cytometer correlates the side scatter diffusion of rays and forward scatter. Various parameters available on the cytometer are used for gating based on the application selected by the researcher. Flow cytometer can detect optical and fluorescence parameters of single cell size, internal complexity and the fluorescence of the chosen cells is analysed to distinguish the positively stained cells with the unstained cells. Fluorescent dyes can bind to so many various components, along with antibodies different fluorescent dyes conjugated and can bind on plasma membrane/inside cells. When labelled cells are made to passed through a light, they fluorescence. By the use of multiple fluoro-chromes, each with same excitation, having different emission wavelengths, many cell properties are measured simultaneously. In this study we have used propidium iodide, phycoerythrin, and fluorescein; several other dyes are also available.

2.5. Evaluation

Table 1: Age group and gender wise distribution of thrombocytopenia patients screened

Age Group (yrs)	Males		Females		Total	Total
	No. of males	%	No. of females	%	No. of cases	%
<20	07	14	06	12	13	26
21-30	06	12	08	16	14	28
31-40	03	06	08	16	11	22
41-50	03	06	03	06	06	12
>50	03	06	03	06	06	12
Total	22		28		50	100

Interpretation - In the present study maximum cases are lying between the age group of 21 to 30 years, followed by < 20 years age group.(Table 1)

Table 2: Distributions of CD42b, CD41, CD61, CD62P among cases

Antibody	No. of cases Positive	No. of cases Negative	Total cases
CD 42b	02	48	50
CD 41	06	44	50
CD 61	06	44	50
CD 62p	04	46	50

Interpretation - 02 (4%) cases show CD 42b, 06 (12%) cases show CD41, 06 (12%) cases show CD 61 and 04 (8%)

cases show CD 62p.(Table 2)

Table 3: Ages and gender variation in immunological and non-immunological causes of thrombocytopenia

Age Group(yrs)	Immunological Thrombocytopenia		Non-immunological Thrombocytopenia	
	Males	Females	Males	Females
<20	01	01	06	05
21-30	01	-	05	08
31-40	-	01	03	07
41-50	-	01	03	02
>50	-	01	03	02
	P -0.08		P-0.26	

Interpretation - The present study, comprised of 50 cases, out of this 06(12%) were immunological thrombocytopenia & 44 (88%) were non-immunological. (Table 3)

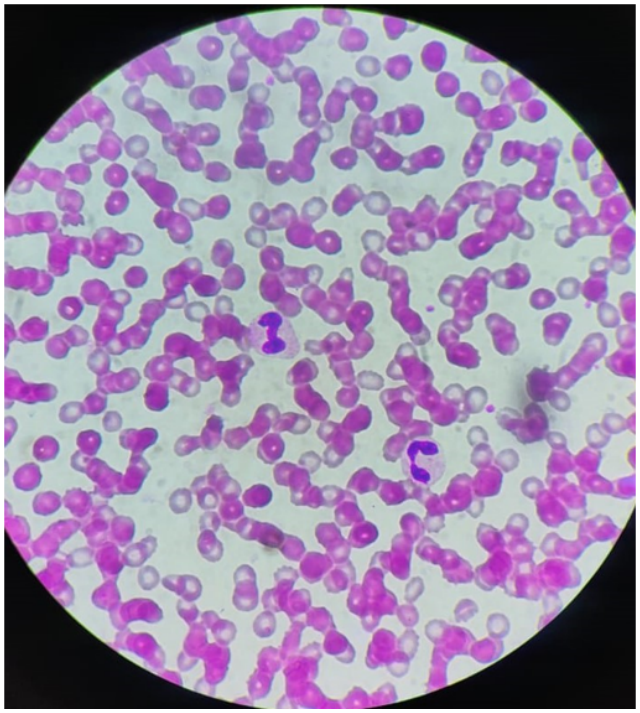

Figure 1: Peripheral smear showing thrombocytopenia

3. Results

In present study maximum cases are lying between the age group of 21 to 30 years, followed by < 20 years age group. p-0.60 & chi square value-1.85 which is insignificant indicate that distribution of thrombocytopenia is similar in males and females in different age groups.

In this study 02 (4%) cases show CD 42b, 06 (12%) cases show CD41, 06 (12%) cases show CD 61 and 04 (8%) cases show CD 62p.p- 0.44, chi square value-2.68 which is suggest that distribution of antibodies against cd42b, cd41, cd61, cd62p among cases is equal.

The present study comprised of 50 cases, out of this 06(12%) were immunological thrombocytopenia & 44 (88%) were non immunological. The number of non-immunological patients was more than immunological patients. Statistics – P-value in group with immunological thrombocytopenia is 0.08 which is nearly significant in above 30 age group means in above 30 age group immunological thrombocytopenia is more common in females in non-immunological group P-value is 0.26, which is insignificant. (p-value<0.05 is significant). Suggest that non-immunological thrombocytopenia is equally distributed among males and females.

4. Discussion

In 1968 - Wolfgang Göhde - the University of Münster, Germany, developed first fluorescence device and the first commercialization was done in 1968 by German developer and manufacturer Partec through Phywe AG in Göttingen. Previously absorption-based methods were widely used by scientists over fluorescence but after than it become most favoured one.[14] In this study Flow cytometer analysed the blood sample for platelet recepters adjust the FITC and PE signals so that the intensity of fluorescence is a biparametric FITC histogram versus PE.[15] In our study we find out 06 cases having anti platelet antibodies causing thrombocytopenia, further more number of cases needed to be studied to support the prevalence. In 2010, Stellos K and Bigalke B, studied Platelet-bound P- selectin expression, in the patients of coronary artery diseases and assessed that the effect of myocardial necrosis, diabetes mellitus or anti- platelet medication on P-selectin. And found it is as a prognostic marker in myocardial infarction (MI), and found a positive correlation between the level of myocardial injury and CD62P positivity, regardless of age and sex. This study also supports our findings and in our study, we have 04 cases having cd62p positivity.[16]

5. Conclusion

We studied 50 thrombocytopenia cases out of which 06(12%) were immunological thrombocytopenia & 44 (88%) were non-immunological thrombocytopenia. In our study we have 04 cases having cd62p positivity.

6. Source of Funding

None.

7. Conflict of Interest

None.

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