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Evaluation of Thrombocytopenia with Special References to Platelet Indices in Five Part Cell Counter

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Abstract

Introduction: Thrombocytopenia is defined as platelet count less than 150,000µl. There are various causes of thrombocytopenia's include increased peripheral destruction of platelets, decreased production of platelet, increased splenic sequestration abnormal platelet production. Platelet indices including platelet count, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) & large platelet cell ratio (P-LCR) are helpful in evaluation of thrombocytopenias. Present study is evaluating this platelet indices by using five part cell counter.

Method: It was an observational cross-sectional study conducted in Department of Pathology. All detected thrombocytopenia cases (platelet countless than 1.5 lakh/mm3) from CBC done by Five Part Cell Counter.

Results: 150 cases of thrombocytopenia were included in our study. Majority of patients i.e 66.7% were in the age group of 15-49 yrs including males (52.7%) & females (47.3%). Infective conditions were found to be commonest cause of thrombocytopenia with 108 cases (72%), followed by 13(8.7%) anaemia cases, 8 (5.3%) ITP cases. The commonest cause of thrombocytopenia in was dengue with maximum cases of 74 (49.33%) out of 150 cases of thrombocytopenia. The mean MPV differed significantly (mean 9.5685 vs 8.5381) between two categories i.e in factious conditions vs all other conditions (except infectious), as indicated by a p-value of 0.02. The mean platelet distribution width (PDW) was significantly different [Mean ± SD (63.06 ± 23.63vs 44.00 ± 23.79)] between two categories i.e in factious conditions and all other conditions (except in fectious) as indicated by ap-value<0.0001. The mean for parameter MPC showed significant difference [Mean ±SD (67.56±20.25 vs 53.30±27.57)] between two categories of dengue and other infectious disease with a p-value of 0.033 by using t-test for in dependent variable. The mean P-LCR was significantly higher (0.01 ± 0.01) in infectious condition as compared to non infectious category (0.004±0.008), as indicated by p-value of 0.007. The mean MP Md if fered significantly between two categories of infectious vs non infectious conditions as indicated by a p-value of 0.03.

Conclusion: Newer platelet parameters are being studied so as to ease the prognosis and diagnosis of patients. These parameters are easily available in five part hematological analyser. Parameters like MPV, PDW, MPC, P-LCR & MPM show significant statistical value, thus aiding in diagnosing various causes of thrombocytopenia. PDW & MPM can determine the hypoproductive causes of thrombocytopenia, thus can be utilized for deciding the need of platelet transfusion.

Keyword: Platelet parameters; Thrombocytopenia.

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Introduction

Thrombocytopenia is defined as platelet count less than 150,000 µl. It is a common hematological disorder resulting in bleeding into mucus membranes which presents as petechiae, ecchymoses, epistaxis & gingival bleeding. However, bruising, hematuria, gastrointestinal bleeding and rarely intracranial haemorrhage can also occur.[2,3]

There are various causes of thrombocytopenias include increased peripheral destruction of platelets, decreased production of platelet, increased splenic sequestration abnormal platelet production. Increased peripheral destruction of platelet occurs in ITP, due to drugs and infections. Decreased platelet/defective platelet production occur in bone marrow aplasias, leukemias, myelodysplastic syndrome and inherited disorders.[5]

While evaluating thrombocytopenia in a patient, bone marrow examination has been one of the procedures that have employed since long but procedure is invasive and may not be relevant in immune related thrombocytopenias. Hence, platelet indices which are possible to measure automatically by automated blood cell analysers may be of value in such cases. Platelet indices include platelet count, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), large platelet cell ratio (P- LCR).[12] These indices may be helpful in evaluation of thrombocytopenias. MPV has been proposed to be a potentially useful screening test for platelet activation.[17] Furthermore PDW may reflect change in morphology of platelet due to platelet activation. PCT is crucial measurement of platelet biomass which combines MPV with absolute platelet count. These indices are altered in different clinical conditions like cardiovascular (CV) disease, infectious diseases including tuberculosis, malarial infection, dengue fever and immune related conditions.[13] P LCR were more reliable markers for distinguishing hyper destructive thrombocytopenia from hypo productive thrombocytopenia.[18]

Even though there are many studies on thrombocytopenia, very few studies are done on five-part cell[14,15] counter by studying newer platelet parameters.[16] These newer parameters changes can help in determining prognosis, determining causes of thromobocytopenia and storage of platelets in the body. Hence the need to study these parameters further in detail.

AIMS AND OBJECTIVE

1. To study clinicopathological information to know the common causes of thrombocytopenia in patients visiting tertiary care hospital in Five Part Cell Counter.
2. To study changes in platelet parameters platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (P-LCR), plateletcrit (PCT) and mean platelet component (MPC) in various conditions of thrombocytopenia.

MATERIAL AND METHODS

This was a cross sectional observational study done in Department of Pathology for duration of 2 years. 150 patients of both sexes and all ages who are diagnosed with thrombocytopenia on five part cell counter, attending tertiary care hospital were taken.

Blood samples was collected using ethylenediamine tetra acetic acid bulb (EDTA bulb). Thrombocytopenia cases detected from CBC anaylsed by Five Part Cell Counter was noted. Informed written consent was taken of the patient for research purpose. Complete history, important clinical details was be analysed. Various platelet parameters like MPV, PDW, P-LCR, PCT & MPC were studied in thrombocytopenia cases.

These platelet counts and platelet parameters along with details and history of patients was collected.

Peripheral blood smears and bone marrow aspirations stained with Leishman's stain or Hematoxylin and Eosin stain.

Study instrument

Seimens Advia 2120 Hematology System-Five part hematology analyser.[19] Olympus Cx21i binocular microscope.

Inclusion criteria

All detected thrombocytopenia cases (platelet count less than 1.5 lakh/mm3) from CBC done by 5-part cell counter in department of pathology.

Exclusion criteria

All patients not giving consent.

RESULTS

Table 1 shows the distribution of patients as per age and sex. As regards age, majority, i.e. 100 (66.7%) patients were in the age range of 15-49 years, followed by 24 (16%) in the range 50–64 years, 17 (11.3%) in the 5–14 years. There were 6 (4%) patients above 65 years and 3 (2%) below 5 years. Further, there were 71 (47.3%) females, while 79 (52.7%) males in the study.

Table 2 provides the distribution of patients as per their clinical conditions. There were 108 (72%) cases of infective condition, followed by 13 (8.7%) anaemia cases, 8 (5.3%) peripheral disease cases. Other conditions, as shown in table, were observed in less than 5% of the cases.

Table 1: Distribution of patients according to age and sex

Age Category (years)	Sex		Total
	Number (%)		
	Female	Male	
<5	3 (4.2)	–	3 (2.0)
May–14	8(11.3)	9(11.4)	17(11.3)
15–49	54 (76.1)	46 (58.2)	100 (66.7)
50 – 64	3 (4.2)	21 (26.6)	24 (16.0)
>=65	3 (4.2)	3 (3.8)	6 (4.0)
Total	71 (100)	79(100)	150(100)

Table 2: Distribution of patients as per clinical condition

Clinical Condition	Number (%)
Infective condition	108 (72.0)
Anaemia	13 (8.7)
ITP	8 (5.3)
Epithelial malignancy	3 (2.0)
Haematological malignancy	2 (1.3)
Autoimmune disease	1 (0.7)
Drug toxicity	1 (0.7)
Genetic disorder	1 (0.7)
Pregnancy	1 (0.7)
Others	12 (8.0)
Total	**150 (100)**

Table 3 gives the mean and standard deviation for various platelet parameters according to clinical condition. For statistical comparison of parameters, the conditions like anaemia, peripheral destruction, epithelial malignancy, haematological malignancy, auto immune disease, drug toxicity, genetic disorder, pregnancy and other conditions were merged together due to sample inadequacy. The parameters for this combined group were compared with that of infective condition for significance of difference of means using two sample independent t-test. Table 12A gives the mean and standard deviation for pooled clinical conditions. Table 4 gives the comparison of platelet parameters between two diagnostic categories using t-test for independent samples. It is evident that the mean platelet distribution width (PDW) was significantly different between two categories as indicated by a

Table 3: Mean and Standard deviation for various platelet parameters as per clinical condition

Clinical condition	N	Mean ± SD				
		Platelet (104)	PDW	PCT	Large Platelet	Large Platelet %
Infective condition	108	5.30 ± 2.79	63.07 ± 23.64	0.05 ± 0.03	3.43 ± 2.96	0.01 ±0.01
Anaemia	13	5.01 ± 2.45	35.43 ± 21.34	0.04 ± 0.02	0.62 ± 0.96	–
ITP	8	4.13 ± 3.55	47.34 ± 28.42	0.04 ± 0.07	5.5 ± 14.35	0.01 ±0.02
Epithelial malignancy	3	8.72 ± 2.30	30.1 ± 16.13	0.02 ± 0.02	–	–
Haematological malignancy	2	2.31 ± 1.98	62 ± 25.03	0.04 ± 0.01	1.5 ± 2.12	0±0.01
Autoimmune disease	1	7.9	21.6	0.02	–	–
Drug toxicity	1	0.2	40.5	0.05	–	–
Genetic disorder	1	6.4	21.3	0.04	1	–
Pregnancy	1	9.3	88.5	0.07	7	0.01
Others	12	3.83 ± 2.45	51.89 ± 21.13	0.05 ± 0.03	1.83 ± 2.33	0.01 ±0.01

Table 4: Comparison of platelet parameters between two diagnosis categories

Parameter	Diagnosis category	N	Mean ± SD	P-value*
Platelet (104)	Infectious condition	108	5.30 ±2.79	0.274
	Others (except infectious condition)	42	4.71± 3.00	
PDW	Infectious condition	108	63.06 ±23.63	< 0.0001
	Others (except infectious condition)	42	44.00 ±23.79	
PCT	Infectious condition	108	0.05 ± 0.03	0.206
	Others (except infectious condition)	42	0.04 ±0.04	
Large platelet	Infectious condition	108	3.43± 2.95	0.181
	Others (except infectious condition)	42	2.02± 6.41	
Platelet large cell ratio	Infectious condition	108	0.01 ±0.01	0.007
	Others (except infectious condition)	42	0.004 ± 0.008	

Bold p-values indicate statistical significance; *Obtained using t-test for independent samples.

p-value < 0.0001. The mean was higher in infectious condition as compared to other category. Similarly, the mean large platelet percent was significantly higher in infectious condition as compared to other category, as indicated by p-value of 0.007. The mean was higher in infectious condition. The other parameters showed insignificant difference of means.

Table 5: Mean and standard deviation for mean platelet count, plate volume and platelet mass as per clinical condition

Clinical Condition	n	Mean ± SD		
		MPC	MPV	MPM
Infective Condition	103	27.02 ±1.85	9.57 ±1.94	2.34 ±0.28
Anaemia	13	26.74 ±2.24	7.58 ± 1.35	2.05 ±0.24
ITP	8	27.54 ± 2.17	9.29 ±4.06	2.35 ±0.6
Epithelial Malignancy	3	25.67 ±4.65	7.93 ±1.02	2.07 ±0.51
Haematological Malignancy	2	27 ±3.25	10.3 ±2.97	2.46 ±0.16
Autoimmune Disease	1	28.6	7.1	2.16
Drug Toxicity	1	28.5	7.2	2.04
Genetic Disorder	1	25.6	6.9	1.86
Pregnancy	1	27.9	10.5	2.43
Others	12	26.24 ±2.37	9.14 ± 2.55	2.25 ± 0.47

Table 5 gives the mean and standard deviation for mean platelet count, mean platelet volume and mean platelet mass, according to clinical condition. The statistical comparison of parameters was performed between infective condition and all the remaining conditions merged together.

Table 6: Comparison of parameters between two diagnosis categories

Parameter	Diagnosis category	n	Mean	Std. Deviation	P-Value
MPC	Infectious condition	108	27.0241	1.85013	0.536
	Others (except infectious condition)	42	26.7714	2.36698	
MPV	Infectious condition	108	9.5685	1.94401	0.02
	Others (except infectious condition)	42	8.5381	2.51056	
MPM	Infectious condition	108	2.3447	.28372	0.03
	Others (except infectious condition)	42	2.1895	.41614	

Bold p-values indicate statistical significance; *Obtained using t-test for independent samples

Table 6 shows that the mean MPV differed significantly between two categories, as indicated by a p-value of 0.02. The mean for infectious category was significantly higher than that of the other category. Similarly, the mean MPM differed significantly between two categories as indicated by a p-value of 0.03. The mean for infectious category was significantly higher than that of other category.

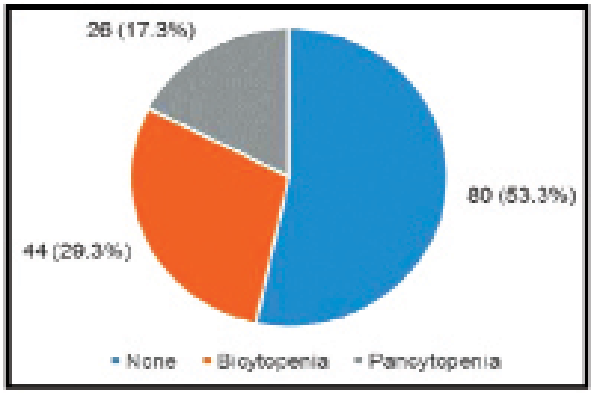

Fig. 1: Pie chart showing distribution of patients as per reduction in cellular elements

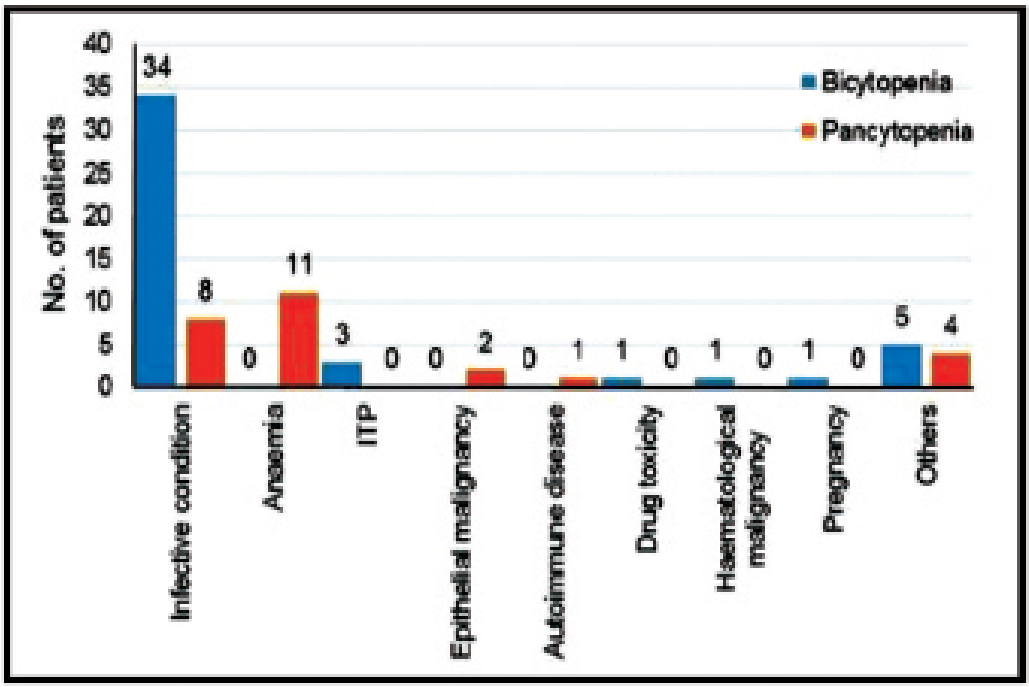

Fig. 2: Column chart showing number of patients as per reduction in cellular elements in each clinical condition

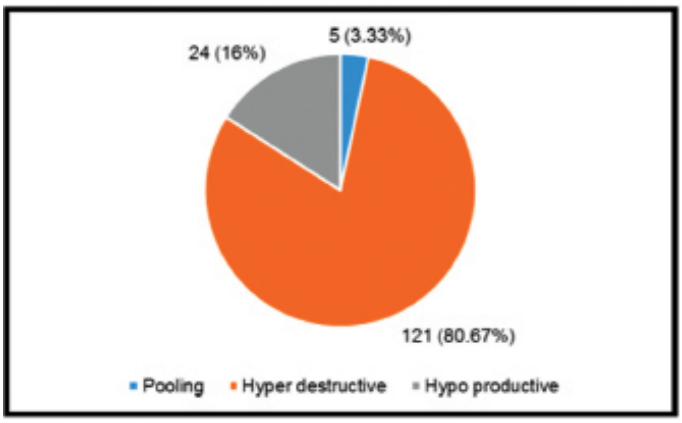

Fig. 3: Pie chart showing distribution of patients as per type of thrombocytopenia.

Table 7: Descriptive statistics for platelet parameters according to type of thrombocytopenia

Type	N	Platelet (O'lO4)	PDW	PCT	Large Platelet	Platelet Large Cell Ratio
Hyperdestructive	12	5.18 ± 2.81	60.44 ±24.45	0.05 ± 0.03	3.32 ± 4.49	0.01 ±0.01
Hypoproductive	24	5.15 ± 3.23	47.63 ± 26.07	0.05 ± 0.02	1.92 ± 2.81	0±0
Pooling	5	3.86 ± 8.79	40.66 ± 21.45	0.05 ± 0.02	1.40 ± 2.61	0±0.01
P-value* (All three types)		0.599	0.021	0.985	0.228	0.161
P-value1: Hyper vs.Hypo		0.999	0.045	0.985	0.300	0.182
P-value1: Hyper vs.Pooling		0.569	0.187	0.997	0.581	0.646
P-value:: Hypo vs.Pooling		0.627	0.833	0.999	0.967	0.999

Bold p-value indicate statistical significance; *Obtained using one-way ANOVA; ‡Obtained using Tukey's post-hoc test

Table 7 gives the mean and standard deviation of platelet parameters according to the type of thrombocytopenia. The comparison of mean values of parameters was carried out using one-way analysis of variance (ANOVA). The mean platelet differed insignificantly across three types (0.599), while mean PDW differed significantly across groups, with a p-value of 0.021. The mean PDW for hyper destructive group was significantly higher than that of hypo and pooling group (p=0.045), as observed using Tukey's post-hoc test. The parameters PCT, large platelet and large platelet % showed insignificant difference of means across three types as well as in paired comparisons.

Table 8: Descriptive statistics for mean platelet count, platelet volume and platelet mass according to type of thrombocytopenia.

Type	n	MPC	MPV	MPM
Hyperdestructive	121	27.09 ±1.92	9.4 ±2.05	2.33 ±0.32
Hypoproductive	24	26.31 ± 2.4	8.68 ±2.2	2.15 ±0.33
Pooling	5	26.72 ± 1.92	9.26 ±4.11	2.31 ±0.58
P-value* (All types)		0.215	0.330	0.054
P-valued Hyper vs. Hypo		0.193	0.296	0.042
P-value*: Hyper vs. Poolmg		0.913	0.989	0.988
P-value*: Hypo vs. Pooling		0.909	0.848	0.599

Bold p-value indicate statistical significance; *Obtained using one-way ANOVA; ‡Obtained using Tukey's post-hoc test.

Table 8 gives the mean and standard deviation for mean platelet count, mean platelet volume and mean platelet mass according to type of thrombocytopenia. The means for all the three parameters differed insignificantly across three types as indicated by p-values >0.05 using one way ANOVA. Pair wise comparison of parameters revealed that the difference between hyper and hypo was significant with a p-value of 0.042. However, all other paired differences were statistically insignificant.

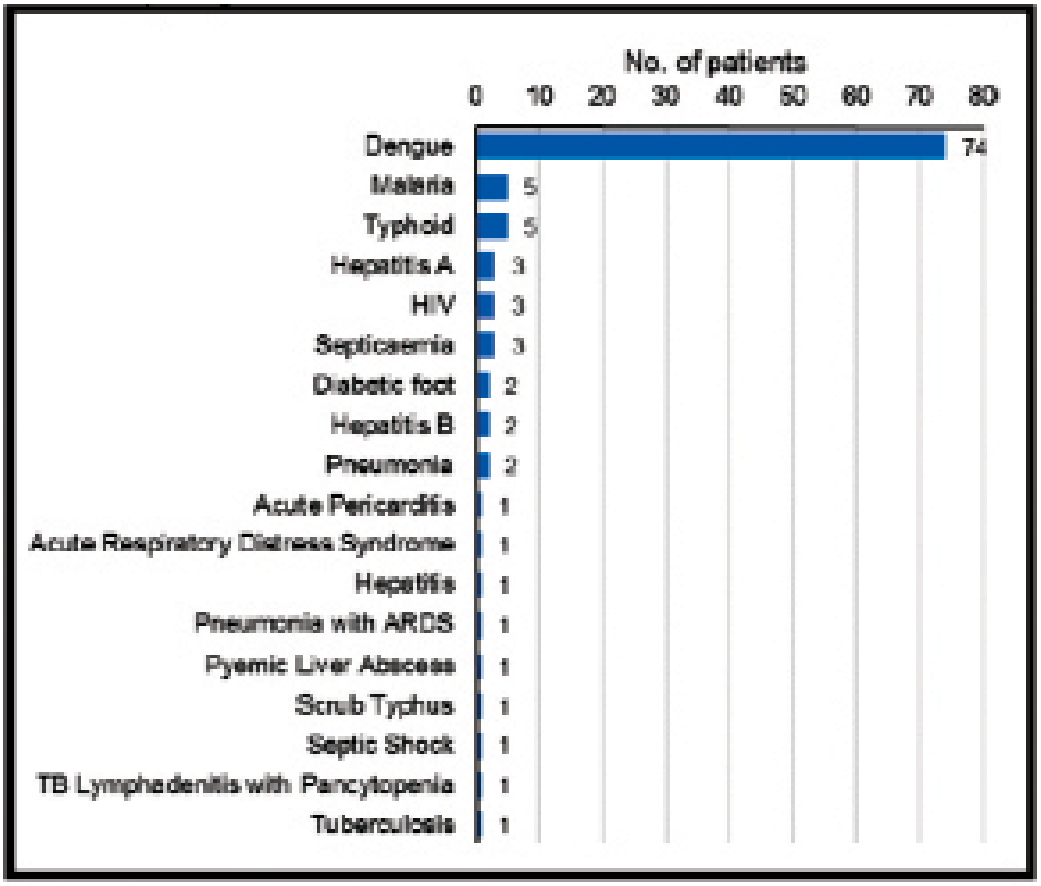

Fig. 4: Horizontal bar chart showing number of patients as per diagnosis – Infectious condition.

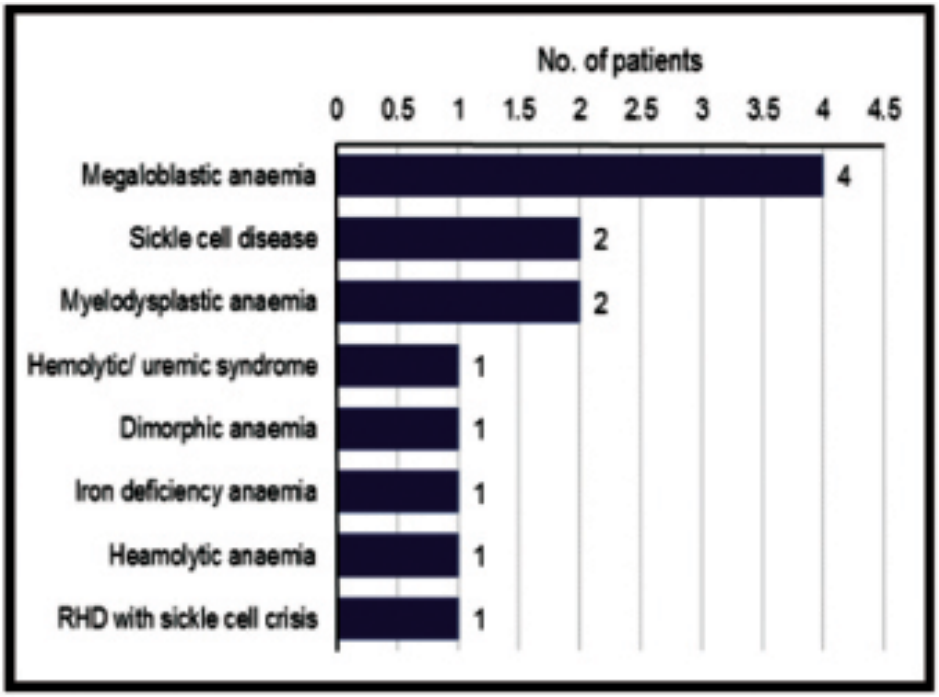

Fig. 5: Horizontal bar chart showing number of patients as per diagnosis – Anaemic condition

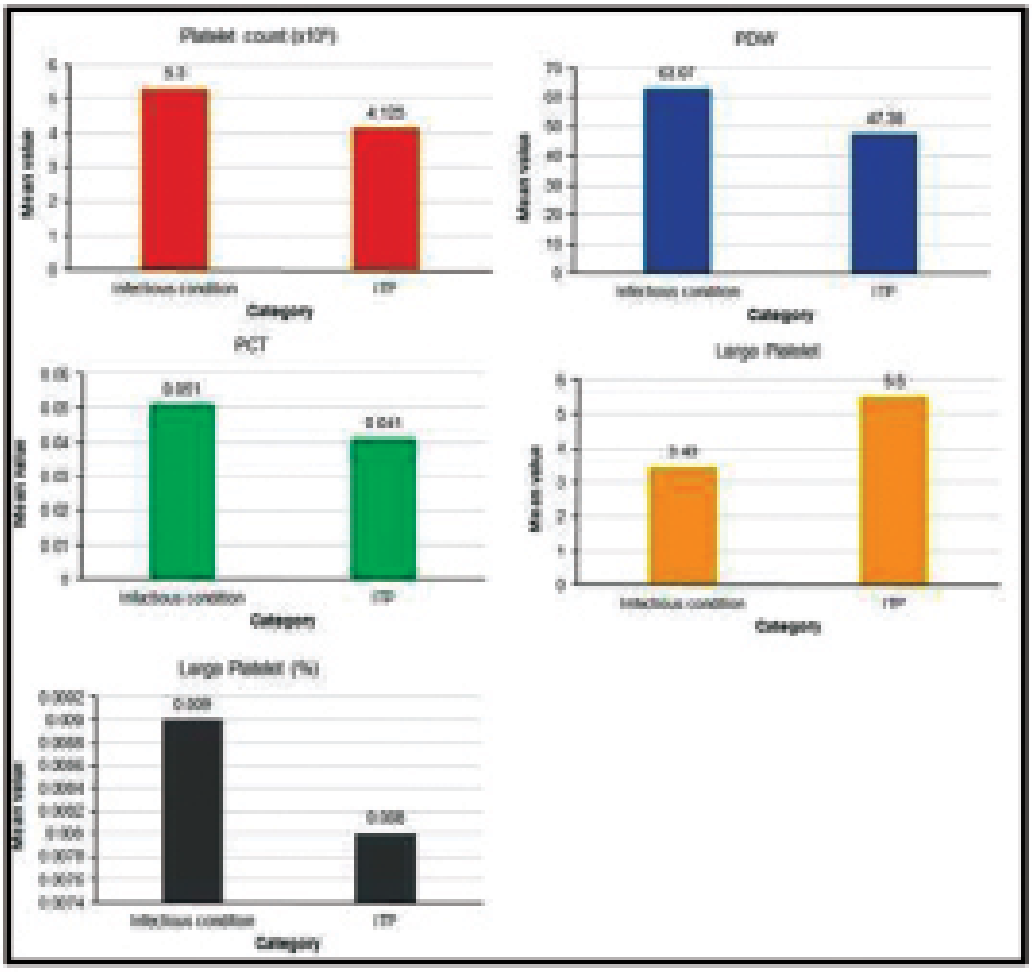

Fig. 6: Column chart showing the mean value of parameters between two diagnostic categories

Table 9: Comparison of parameters between two diagnosis categories

Parameter	Diagnosis Category	N	Mean	Std. Deviation	P-value
MPC	Infectiouscondition	108	27.02	1.85	0.456
	ITP	8	27.54	2.17	
MPV	Infectiouscondition	108	9.57	1.94	0.720
	ITP	8	9.28	4.05	
MPM	Infectiouscondition	108	2.35	0.28	0.989
	ITP	8	2.35	0.60	

*Obtained using t-test for independent samples

Table 9 shows that the parameters MPC, MPV and MPM differed in significantly between twocategories, as indicated by ap-values >0.05

Table 10 gives the comparison of platelet parameters between two diagnostic categories using t-test for independent samples. The mean PDW differed significantly between two diagnostic categories, as indicated by p-value of 0.003. The other parameters showed insignificant difference of means between two categories.

Table 10-Comparisonofplateletparameterbetweentwodiagnosis categories

Parameter	Diagnosis category	n	Mean ± SD	P-value*
Platelet (104)	Dengue	74	5.32±2.76	0.908
	Other infectious condition	34	5.25±2.89	
PDW	Dengue	74	67.56±20.25	0.003
	Other infectious condition	34	53.30±27.57	
PCT	Dengue	74	0.050±0.029	0.833
	Other infectious condition	34	0.052±0.025	
Largeplatelet	Dengue	74	3.65±3.05	0.250
	Other infectious condition	34	2.94±2.73	
Platelet large cell ratio	Dengue	74	0.010± 0.015	0.322
	Other infectiou scondition	34	0.007±0.011	

*Obtained using t-test for independent samples.

Table 11: Comparison of platelet parameters between two diagnosis categories

Parameter	Diagnosis category	N	Mean	Std. Deviation	P-value
MPC	Dengue	74	27.28	1.76	0.033
	Other infectious condition	34	26.47	1.94	
MPV	Dengue	74	9.68	1.88	0.377
	Other infectious condition	34	9.32	2.07	
MPM	Dengue	74	2.36	0.27	0.481
	Other infectious condition	34	2.32	0.31	

*Obtained using t-test for independent sample shows that the mean for parameter MPC showed significant difference between two categories with a p-value of 0.033. The other two parameters i.e., MPV and MPM differed insignificantly between two categories, as indicated by a p-values> 0.05.

DISCUSSION

Age wise distribution

In our study we included 150 cases of thrombocytopenia. The age group ranged from neonates to elderly. Majority of patients i.e 66.7% were in the age group of 15-49 yrs, followed by 16% in the range 50 – 64 years, 11.3% in the 5 – 14 years. There were 4% patients above 65 years and 2% below 5 years. The youngest was 5 day old and the oldest was 70 year old.

Sex wise distribution

In our study cases of thrombocytopenia were more in males (52.7%) than in females (47.3%).

Clinical condition wise distribution of thrombocytopenia.

In our study infective conditions were found to be commonest cause of thrombocytopenia with 108 cases (72%), followed by 13 (8.7%) anaemia cases, 8 (5.3%) ITP cases. Epithelial malignancy, haematological malignancy, other autoimmune

disease, drug toxicity, genetic disorder, pregnancy & others (which included liver cirrhosis, heamorrahges, head trauma & CVE) formed 21 (14.2%) of the total cases as stated in table 11. In infective conditions, maximum cases were found in dengue infection i.e 74 (68.5%), followed by malaria infection & typhoid with each having 5 (4.63%) cases. There were 3 (2.78%) cases each of hepatitis A, HIV and septicaemia, followed by 2 (1.85%) cases each of diabetic foot, hepatitis B, pneumonia and single cases of other diagnosis as shown in the figure 4.

In our study the commonest cause of thrombocytopenia in was dengue with maximum cases of 74 (49.33%) out of 150 cases of thrombocytopenia. The reason for dengue to be the commonest cause of thrombocytopenia was presence of regional epidemic of dengue during the period of study.

Classification of thrombocytopenia cases

Cases of thrombocytopenia were further classified into3categories of hypoproductive, hyperdestructive & pooling respectively, as according to the causes. Table16 gave the distribution of patients as per type of thrombocytopenia. The majority i.e. 121 (80.67%) cases were hyper destructive, followed by 24 (16%) with hypo destructive. There were 5 (3.33%) cases of pooling.

Platelet parameters

Individual platelet parameters like MPV, PDW, PCT, MPC, L-PCR & MPM were studied according to the clinical conditions, distribution & classification of thrombocytopenia cases, to find out their significance.

Mean platelet volume

MPV was studied Table 6 shows that the mean MPV differed significantly (mean 9.5685 vs 8.5381) between two categories i.e infectious conditions vs all other conditions (except infectious), as indicated by a p-value of 0.02. The mean for infectious category was significantly higher than that of the other category. Thus our study signified that MPV helps in differentiating infective conditions from non infective conditions.

Platelet distribution width

It was evident that the mean platelet distribution width (PDW) was significantly different [Mean ± SD (63.06 ± 23.63vs 44.00 ± 23.79)] between two categories i.e infectious conditions and all other conditions (except infectious) as indicated by a p-value < 0.0001. The mean was higher in infectious condition as compared to other category as stated in table 10 based on t-test for independent samples. Our study showed increased in PDW in dengue disease as compared to other infectious disease. There was no statistical significance of PDW in differentiating ITP and infectious conditions as indicated in figure 6.

Platelet crit

No stastistical significance was found in PCT in differentiating clinical condition and causes of thrombocytopenia.

Mean platelet component

Mean platelet component is a measure of mean refractive index of the platelets & correlate with the platelet activation state. It has become a potential predictive parameter for acute ischemic complications or thrombotic risk.[20]

However in our study the mean for parameter MPC showed significant difference [Mean ± SD (67.56± 20.25 vs 53.30± 27.57)] between two categories of dengue and other infectious disease with a p-value of 0.033 in table 11 by using t-test for independent variable. Thus mean platelet component can help in distinguishing dengue from other infectious conditions. There is no literature stating the increase in MPC in relation to dengue fever,however Ojha A et al. (2017) stated that low platelet counts coexisted with the high platelet activation and vice versa during different days of infection in dengue patients.[21]

Platelet large cell ratio (large platelet percentage)

The mean P-LCR was significantly higher (0.01 ± 0.01) in infectious condition as compared to non infectious category (0.004 ± 0.008), as indicated by p-value of 0.007. The mean was higher in infectious condition as indicated in table 10. Thus large platelet cell ratio can help us in distinguishing causes of thrombocytopenia from infective and non infective conditions. No statistical significance was established in our study so as to differentiate hypoproductive vs hyperdestructive causes of thrombocytopenia by studying P-LCR. Large platelets cell increases in the late stage of hyperdestructive cause of thrombocytopenia. So if the parameter was studied in early stage of diseases (at the time of diagnosis), large platelet cell would'nt have been significantly changed. Hence probably due to this, significance of L-PCR was not found in our study. And for this, purpose sequential changes of platelet parameters will be

helpful to appreciate significance in L-PCR.

Mean platelet mass

The mean MPM differed significantly between two categories of infectious vs non infectious conditions as indicated by a p-value of 0.03 in table 6. Thus MPM can distinguish infective causes of thrombocytopenia from non infective causes. However no similar literature was found comparing MPM in infectious and non infectious conditions.

Pair wise comparison of MPM revealed that the difference between hyperdestructive (mean +/- SD2.33 ± 0.32) and hypoproductive causes (mean +/- SD2.15 ± 0.33) was significant with a p-value of 0.042. Thus MPM can help in differentiating causes of hyperdestructive thrombocytopenia from hyperproductive.

CONCLUSION

Thrombocytopenia can be due to various causes and is present in various clinical conditions. Thrombocytopenia is more common in males than females. The commonest age group for thrombocytopenia is 15-45 yrs. Thrombocytopenia can be associated with other cytopenias. Dengue is one of the most common infective cause of thrombocytopenia. Megaloblastic anaemia is commonest causes of thrombocytopenia due to decrease platelet production.

Newer platelet parameters are being studied so as to ease the prognosis and diagnosis of patients. These parameters are easily available in five part hematological analyser. Parameters like MPV, PDW, MPC, P-LCR & MPM show significant statistical value, thus aiding in diagnosing various causes of thrombocytopenia. PDW & MPM can determine the hypoproductive causes of thrombocytopenia, thus can be utilised for deciding the need of platelet transfusion.

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