"UTILITY OF PLATELET INDICES IN THROMBOCYTOPENIA AND ITS CORRELATION WITH BLEEDING TENDENCY"

BY

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DISSERTATION SUBMITTED TO
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IN

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UNDER THE GUIDANCE OF

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PROFESSOR AND HEAD OF DEPARTMENT,

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SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

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LIST OF ABBREVIATIONS

ADP	**-**	**ADENOSINE DIPHOSPHATE**
AML	**-**	**ACUTE MYELOID LEUKEMIA**
ANOVA	**-**	**ANALYSIS OF VARIANCE**
APTT	**-**	**ACTIVATED PARTIAL THROMBOPLASTIC TIME**
ATP	**-**	**ADENOSINE TRIPHOSPHATE**
BSS	**-**	**BERNAURD SOULIER SYNDROME**
CFU	**-**	**COLONY FORMING UNIT**
CMP	**-**	**COMMON MYELOID PROGENITOR**
CMV	**-**	**CYTOMEGALOVIRUS**
DG	**-**	**DENSE GRANULES**
DHF	**-**	**DENGUE HEMORRHAGIC FEVER**
DIC	**-**	**DISSEMINATED INTRAVASCULAR COAGULATION**
DNA	**-**	**DEOXYRIBONUCLEIC ACID**
EDTA	**-**	**ETHYLENE DIAMINE TETRA ACETIC ACID**
GP	**-**	**GLYCOPROTEIN**
GI	**-**	**GASTROINTESTINAL**
HB	**-**	**HEMOGLOBIN**
HIV	**-**	**HUMAN IMMUNODEFICIENCY VIRUS**
HSV	**-**	**HERPES SIMPLEX VIRUS**
IgG	**-**	**IMMUNOGLOBIN G**
IL-6	**-**	**INTERLEUKIN 6**
INR	**-**	**INTERNATIONAL NORMALIZED RATIO**
IPF	**-**	**IMMATURE PLATELET FRACTION**
ITP	**-**	**IMMUNE THROMBOCYTOPENIC PURPURA**

I/V - INTRAVENOUS

MPV - MEAN PLATELET VOLUME

NM - NOT MENTIONED

PDW - PLATELET DISTRIBUTION WIDTH

PE - PHOSPHADITYL ETHANOLAMINE

PI - PHOSPHATIDYL IONOSITOL

PLCR - PLATELET LARGE CELL RATIO

PS - PHOSPHATIDYL SERINE

RAI - RADIOACTIVE IODINE

RES - RETICULOENDOTHELIAL SYSTEM

SLE - SYSTEMIC LUPUS ERTHYMETOSUS

TSH - THYROXINE STIMULATNG HORMONE

TTP - THROMBOTIC THROMBOCYTOPENIC PURPURA

VWF - VON WILLIBRAND FACTOR

ABSTRACT

TITLE OF THE STUDY:

"UTILITY OF PLATELET INDICES IN THROMBOCYTOPENIA AND ITS CORRELATION WITH BLEEDING TENDENCY"

INTRODUCTION

Patients with platelet count <1.5 L/cumm are at risk of bleeding but, however not all patients with thrombocytopenia bleed. There is no linear correlation with platelet count and bleeding risk.

So in the current study, use of platelet indices for assessing bleeding tendency is done. Platelet indices includes- mean platelet volume, plateletcrit and platelet distribution width. It's seen that patients with bleeding will usually have low platelet indices compared to the thrombocytopenic patients without bleeding. Bleeding include- conjunctival bleed , oropharyngeal bleed, hemoptysis, melena , epistaxis, petechiae, bleeding from venipuncture sites.

Our study included all adult patients with age > 18 years male / female with platelet count < 1.5 lakh/cumm. Patients with abnormal PT and aPTT will be excluded.

OBJECTIVES OF THE STUDY

1) To study platelet indices in patients with thrombocytopenia.

2) To assess bleeding tendency in thrombocytopenic patients with respect to platelet indices.

MATERIALS AND METHODS

Study was done on 190 patients with thrombocytopenia, 95 with bleeding and 95 without bleeding.

All patients with platelet count < 1.5 Lakhs/cumm were studied, assessed for bleeding tendency and platelet indices-Mean platelet volume (MPV), plateletcrit, platelet to large cell ratio (PLCR) and platelet distribution width (PDW) was compared in patients with bleeding and without bleeding.

RESULTS:

In our study of 190 patients of thrombocytopenia, 95 with bleeding and 95 without bleeding, we found that: Majority of patients with platelet count <20,000/cumm and 20,000-50,000/cumm presented with bleeding with infectious etiology and bleeding was more common with lower platelet count. Mean platelet count in patients who presented with bleeding was 44,752.6/cumm and among subjects without bleeding subjects was 66,268.8/cumm. In patients with bleeding, low platelet indices including MPV, PDW, Plateletcrit and PLCR was noted. MPV, PDW PLCR and plateletcrit had significant positive correlation in patients of thrombocytopenia with bleeding. Mean PDW among those with bleeding was 15.2 ± 7.9fL and without bleeding was 18.9 ± 6.2fL. Mean PLCR among those with bleeding was 24.78 ± 5.87% and without bleeding was 27.07 ± 9.28%.

CONCLUSION:

Alterations in platelet indices helps predict the bleeding tendency. In patients with thrombocytopenia with bleeding, platelet indices (MPV, PDW, Plateletcrit and PLCR) were low whereas, in patients with thrombocytopenia without bleeding, higher platelet indices were noted.

KEY WORDS:

Mean platelet volume, Plateletcrit, Platelet distribution width, Platelet to Large Cell Ratio (PLCR).

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INTRODUCTION

Bleeding tendency is not only dependent on platelet count, but other factors such as vascular status, platelet function, anticoagulant medications and other plasma factors involved in coagulation.

Thrombocytopenia is defined as platelet count <1.5 lakh/cumm. Patients with platelet count between 1 lakh – 1.5lakh/cumm have no clinical significance. Platelet count < 1 lakh/cumm are associated with clinical complications. Platelet count <50,000/cumm is associated with petechiae, menorrhagia and bruising. Spontaneous bleeding is associated with platelet count <20,000/cu mm and platelet transfusion is indicated. Bleeding depends - medications, blood vessel status, platelet function or the concurrent disease.[1]

Platelet indices include- Mean platelet volume (MPV), Platelet distribution width (PDW), Plateletcrit and PLCR (Platelet to Large Cell Ratio). Mean platelet volume is measurement of average size of platelets. MPV value increases as the platelet count decreases. Platelet distribution width determines the variation in platelet size in peripheral blood smear. Plateletcrit is the ratio of total number of platelets in the blood. The cause of thrombocytopenia can be either decreased platelet production or increased platelet destruction. Decreased platelet production can be due to megakaryocyte hypoplasia or ineffective megakaryopoiesis or inherited disorder. Increased platelet destruction can be seen in immune thrombocytopenic purpura, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome and drugs like heparin, quinine.[1, 2, 3]

Thus, platelet indices like MPV, Plateletcrit, PDW and PLCR has been shown to be useful in predicting the bleeding tendencies of patients with thrombocytopenia. MPV is useful in the detection of platelet production states.[3]

Early diagnosis and treatment of febrile illness with thrombocytopenia will prevent the complications like renal failure, hepatic dysfunction, acute respiratory distress syndrome and severe bleeding.[4]

However, low platelet counts, lower MPV and lower Plateletcrit values are associated with bleeding.[5.6]

NEED OF THE STUDY

Platelet indices includes- Mean platelet volume, platelet distribution width, plateletcrit and PLCR(platelet to large cell ratio) is a non invasive method to detect thrombocytopenia and platelet function.[2]

Platelet count and bleeding has no linear co- relation hence, platelet indices has been studied to assess the risk of bleeding in thrombocytopenic patients.[2, 3]

As there is paucity of literature on relationship between platelet indices and bleeding, this study was undertaken to address the utility of platelet indices in predicting the bleeding tendency of patients with thrombocytopenia.

AIMS AND OBJECTIVES OF THE STUDY

1) To study platelet indices in patients with thrombocytopenia.

2) To asses bleeding tendency in thrombocytopenic patients with respect to platelet indices.

REVIEW OF LITERATURE:

Platelets are disc shaped, non-nucleated fragment with half-life of 4 days. Around 70% of the platelets extruded from bone marrow are in circulating blood whereas, rest 30% in spleen.

PLATELET STRUCTURE:

Divided into 4 regions- peripheral zone, structural zone, organelle zone and membrane system.

1. **Peripheral zone:** It consists of outer surface coat called glycocalyx and inner plasma membrane.

   Glycocalyx: 14-20mm thick, consist of glycolipids, glycoproteins, mucopolysaccharides and plasma proteins.it maintains negative charge on platelet surface that prevents platelet from interacting with each other and with negatively charged endothelial cells.[1]

   Plasma membrane: It consists of bilayer of phospholipid, cholesterol and integral proteins. It maintains cytoplasmic integrity and mediates interaction between platelet and vasculature and plasma proteins.[1]

   Outer membrane of lipid bilayer contains negatively charged phospholipids, phosphatidyl serine (PS), phosphatidyl inositol (PI) and phophatidyl ethanolamine(PE) and inner half of bilayer contains neutral phospholipids-phophatidyl choline and sphingomyelin.[1]

Phospholipid asymmetry is maintained in resting platelets by an ATP dependent amino phospholipid translocase that pumps PE and PS from outer to inner leaflets.

PE and PS accelerates several steps in coagulation sequence and placement of these phospholipids on inner bilayer separates them from plasma coagulation proteins and thus prevents inappropriate coagulation. During platelet activation, these phospholipids move to the outer surface due to activation of scramblase.[1]

Integral proteins- around 30 proteins have been identified.

Membrane receptors: Gp Ib /IX, Gp IIb/ IIIa, Gp Ia/ IIa, GPV

Gp Ib/IX is the most important platelet receptor for VWF and it is found as a complex with Gp IX and GPV in the ratio of 2:2:1.[1]

Glycoprotein Ib- contains two polypeptide chains alpha and beta.GP1b a chain is larger and contains binding sites for VWF, thrombin and ristocetin. The binding sites are located on extracellular portion of GP1b called Glycocalicin. Glycocalicin differentiated thrombocytopenia due to decreased platelet production (low plasma glycocalicin) or due to increased platelet destruction (elevated plasma glycocalicin). Both the alpha and beta chains are associated with the actin binding filamin. GP1b/IX helps in the platelet adhesion through VWF.[1]

GP IIb/IIIa is the most important platelet receptor for fibrinogen. This complex also binds with VWF, thrombospondin, vitronectin and fibronectin.

GP IIb is a two chain protein. The alpha chain is present in the phospholipid bilayer and the beta chain protrudes from the platelet surface. GP IIIa is a single chain phospholipid and complex with GP IIb. This complex is inactive in resting platelets, on activation (by agonists) GP IIb/IIIa is converted into high affinity ligand binding conformation which helps in platelet aggregation process.[1]

2. **Structural zone:** It consists of microtubules (tubulin), microfilament (actin) and intermediate filaments.[1]

   Microtubules: Largest cytoskeletal protein – 5mm diameter consisting of protein tubulin. It maintains the discoid shape of platelets.[1]

   Microfilaments: It consists of protein actin. Actin is present throughout the cytoplasm. It is associated with myosin and other cytoskeletal proteins and the ratio of actin: myosin is 100:1 unlike smooth muscle 7:1. On platelet activation, actin will reorganise, polymerise and form the characteristic pseudopods seen in platelets.

3. **Organelle zone:** Present just beneath the microtubule layer. It consists of mitochondria, glycogen and 4 types of granules dispersed within the cytoplasm- dense granules, alpha granules, lysosomes and peroxisomes.[1]

   Dense granules (DG) – They are so called as they appear dense on electron microscope due to high Calcium content. Platelet contains 3-8 electron dense granules each 20-30nm in diameter. It contains ADP, ATP other nucleotides, phosphate

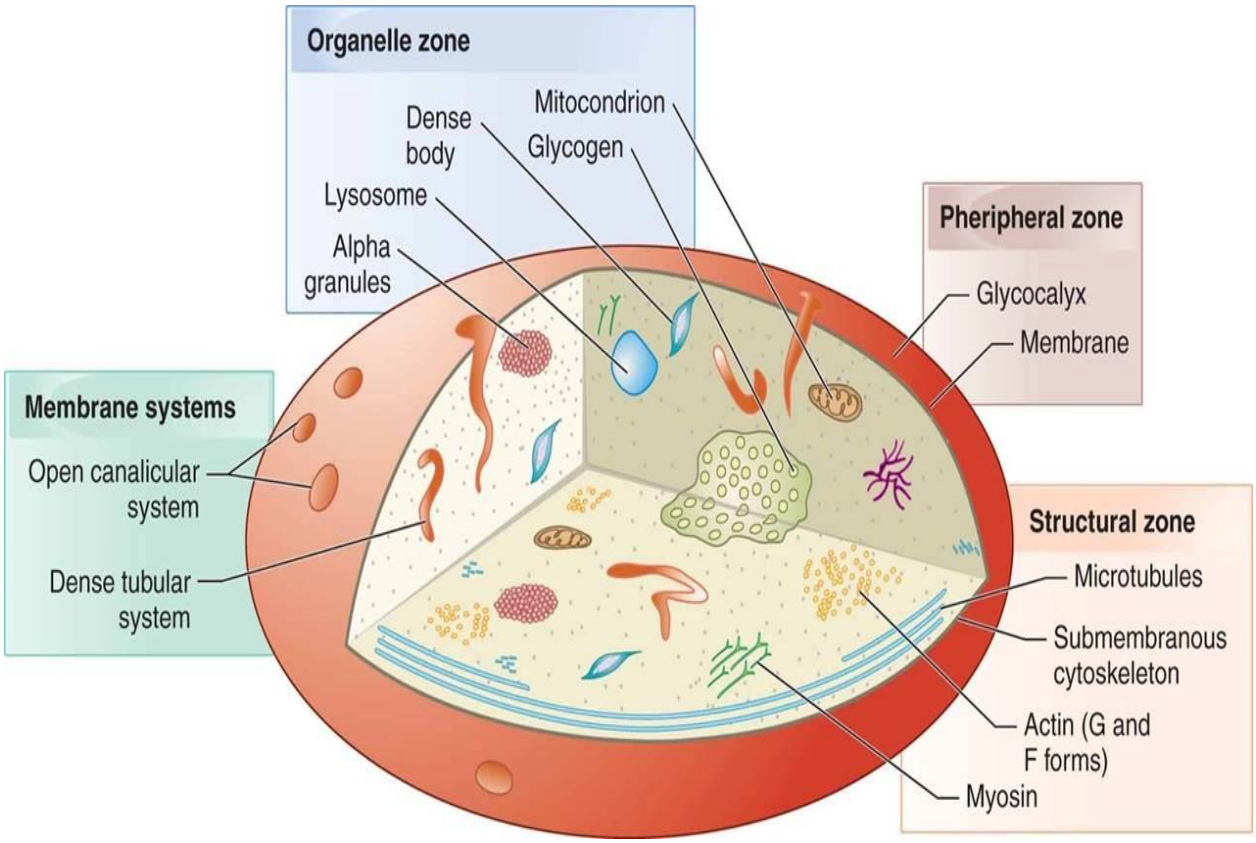
compounds, Calcium and serotonin. ADP: ATP ratio in DG is higher (3:2) than that found in cytoplasm (1:8)[1]

Alpha granules: Most numerous of the four granules. Platelet contains 50-80 alpha granules. They contain two group of proteins- group 1consits of proteins similar to haemostatic proteins (coagulation factors, inhibitors), found in the plasma. Group 2 includes proteins that have variety of functions.[1]

Lysosomes and peroxisomes: Activated platelet releases lysosomes but their release is slower and less complete than release from alpha and dense granules. Peroxisomes are involved in lipid metabolism.[1]

4. **Membrane systems:** It consists of open canalicular system and dense tubular system.[1]

Figure: 1 Structure of platelets



PLATELET COUNT: It is measured by Automated haematological analyser by Impedance method. Normal platelet count was 1.5 – 4.5 lakh/cumm.

PLATELET INDICES: It includes mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit and platelet to large cell ratio (PLCR).

MPV (Mean Platelet Volume) is the measure of platelet size as it is calculated by sum of volumes of platelets divided by platelet count and gives an idea about platelet function.

Increased MPV indicates increased platelet diameter. [7] The platelet distribution curve is used to derive the MPV.

With impedance counting, EDTA causes platelet swelling thus increasing the MPV by 7.9% within 30 min and an overall increase of 13.4% over 24hrs, and the majority of this increase in MPV occurs within the initial 6hrs. Conversely, when MPV is measured by an optical light scatter system derived from the modal platelet size, the MPV decreases over time, possibly because of the dilution of cytoplasmic contents leading to a decrease in light scatter.

MPV is an independent predictor of bleeding and a surrogate marker of bone marrow activity. A raised MPV indicates an increase in the megakaryocyte activity. A reduced MPV indicates bone marrow suppression and a heightened risk of bleeding. Correlation of platelet count and MPV with bleeding and severity of the disease can potentially predict the outcome. Platelet activation leads to activation of coagulation cascade and plays an important role in the pathogenesis of vascular disease – atherosclerosis- coronary artery. [8, 9]

Larger platelets have greater prothrombotic potential and are enzymatically more active than smaller platelets as the number of granules are more in larger platelets thus; the chance of bleeding is decreased in larger platelets as compared with the smaller platelets. Increased MPV predicts the risk in certain conditions such as- Alzheimer's disease, Familial Mediterranean fever and Behcets disease and MPV is found to be low in Endometriosis.[8]

MPV levels depends on thyroid hormone and thyroid hormones relates with platelet function. MPV is increased in hyperthyroid patients and when these patients undergoes RAI ablation therapy, MPV level decreases. MPV level correlate with TSH level except for in autoimmune

conditions.[9] Hou J et al concluded increased MPV is directly related to fasting glucose and glycated Hb rather than insulin resistance or duration of diabetes.[10]

According to a study done on association of MPV with severity, serology and treatment outcome in dengue fever: prognostic utility, the severity of the disease is predicted by decreasing platelet counts. Platelet activation is indicated by MPV and it indirectly tells about the bone marrow activity. But no significant correlation was found between MPV and severity of the disease, thus MPV does not serves as a prognostic parameter in dengue fever.[11]

PDW (Platelet Distribution Width) - Tells us about the variation of the platelet size.

Increased PDW suggests large range of platelet size due to swelling, immaturity or increased platelet destruction.[9]

PDW also calculated from the platelet distribution curve.

PDW and P-LCR are reliable marker for identifying the cause of thrombocytopenia whether due to increased destruction or decreased production. An increased PDW without any change in the MPV may indicate that the platelet anisocytosis index is a more sensitive marker for the estimation of platelet size variation. PDW may be useful in an early detection of pathological conditions as bacteraemia, schistocytosis, platelet consumption or aggregation. On the other hand, an elevated MPV may indicate platelet consumption and activation, which may take place in conditions like disseminated intravascular coagulation.

According to another study on - Platelet size distribution width and mean platelet volume have sufficient sensitivity and specificity in the diagnosis of immune thrombocytopenia they

concluded that though there are many causes of thrombocytopenia, aetiology may vary in given clinical setting. However, viral infections, drugs are responsible for the majority of cases.

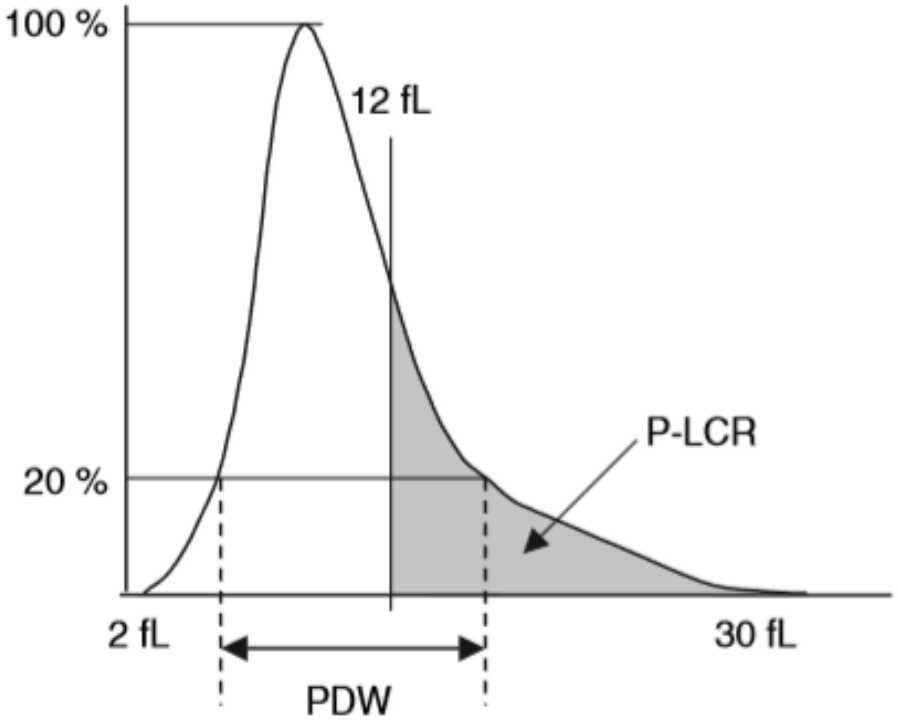
Vinholt P J et al studied platelet indices and methods for evaluating platelet function in thrombocytopenic patients, and concluded that clinical utility of MPV has high predictive value for bone marrow failure as a cause of thrombocytopenia and low MPV is usually associated with bleeding when platelet <20,000/cu mm. PDW gives no information in relation to bleeding risk in thrombocytopenia.[12]

Plateletcrit- is the ratio of total blood platelets in the blood.[9] Like packed cell volume for RBC, plateletcrit measures the total platelet mass.

P-LCR (Platelet to large cell ratio) - is the percentage of platelets with a size of >12fL

PLCR is linked to MPV and more sensitive than MPV in regard to any change in platelet size.

Figure: 2 Platelet indices



Apart from platelet indices, bleeding tendency also depends on platelet functions. Various platelet function tests: [12]

1) Platelet aggregometry-

   It helps to detect defects in platelet membrane glycoprotein in BSS (Bernard Soulier Syndrome) and Glanzmann's Thrombasthenia as well as defects in platelet granule secretion.

   Platelet aggregometry is based on the principle of Light transmission.

2) Platelet secretion assays:

Usually platelet secretion defects is seen in acquired conditions and tells us about the pathophysiology of thrombocytopenia like in Leukemia, MDS (Myelodysplatic Syndrome)

Platelet secretion assays work on the principle of bioluminescence and it detects platelet secretion defects indirectly by the release of granules by activated platelets which causes platelet aggregation.

3) Flow cytometry:

It detects the defects in platelet membrane glycoprotein and granule defects.

Glycoprotein content predicts the bleeding tendancy. Example –In ITP

(Immune Thrombocytopenic Purpura), the platelet membrane glycoprotein is decreased and they respond less to platelet activation and thus, they have more chance of bleeding.

Flow Cytometry works on the principle of Light scattering which is based on the granularity and size of the cell.

4) Bleeding time and Platelet Function Analyzer-100:

Bleeding time and Platelet function analyser helps to detect the defects in platelet membrane glycoprotein but they are not used to detect platelet function defects.

These two have no correlation with the risk of bleeding and lack sensitivity to determine platelet secretion defects.

5) Cone and platelet analyser:

It aids in the determination of platelet activation including- platelet adhesion, platelet release reaction and aggregation, but its utility in disease diagnosis is poor.

This analyser detects risk of bleeding based on platelet count and the surface coverage.

In ITP surface coverage is more compared to patients with BSS and Glanzmann's thrombasthenia.

6) Viscoelastic method:

This method determines the platelet activation and aggregation but not about the platelet adhesion.

It aids in the diagnosis of coagulopathies as it determines initiation and formation of clot, but not for the platelet function defects.

However, the methods mentioned above are expensive and not cost effective, thus not performed in the present study.

PLATELET PRODUCTION (Megakaryopoiesis):

Site of megakaryopoiesis is bone marrow and platelets are produced from the progenitor cell as erythroid and myeloid lineages- (CMP/ CFU-GEMM). Platelets are fragments of cytoplasm of mature megakaryocyte and the megakaryocyte is the platelet precursor cell.[13-16]

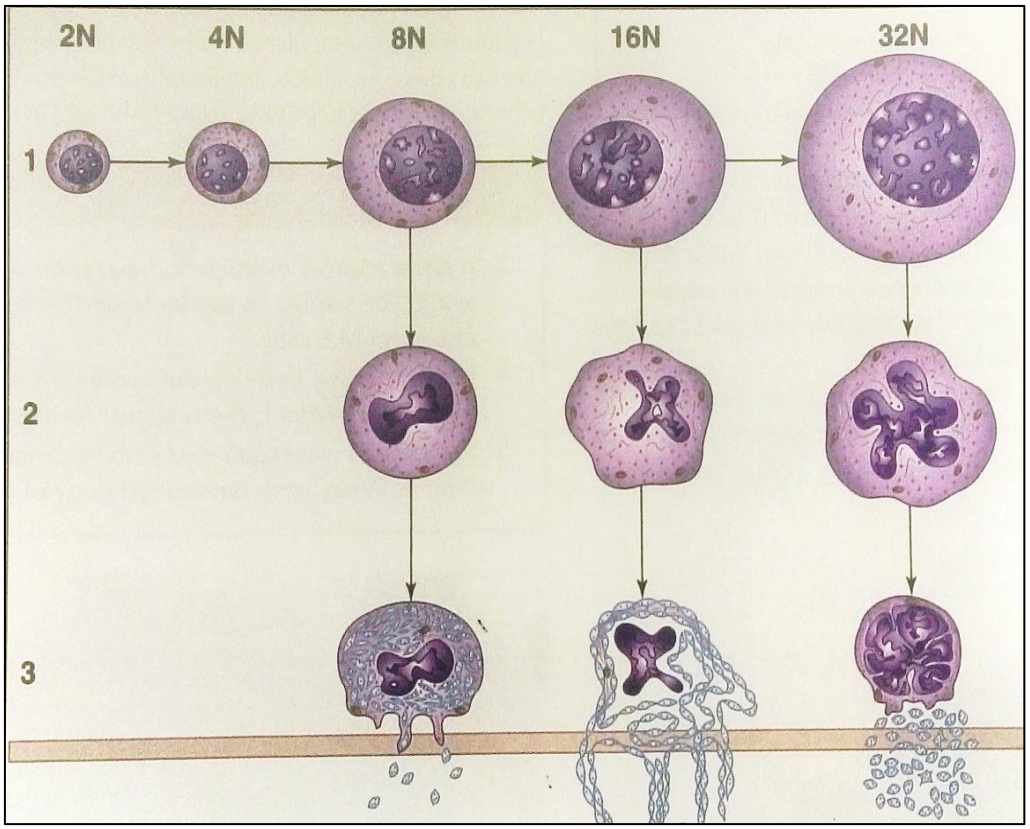
Stages of megakaryocyte development:

Megakaryoblast is the first lineage cell identified not easily by morphology but cytologically by their expression of megakaryocytic specific markers like GP IIb/II a or platelet peroxidase. The megakaryoblast undergoes maturation sequence unlike other marrow lineage where nuclear maturation is followed by cytoplasmic maturation. After the nuclear maturation, cell undergoes endomitosis. Endomitosis is mitosis where the cell DNA content doubles but cell division and nuclear division does not takes place. Series of endomitosis results in polypoidal cells containing increased DNA content within a single nuclear envelope. DNA content of polypoid cells varies from 4N to 64 N. Endomitosis begins in megakaryoblasts and completed in stage II. The first morphologically recognisable stage in bone marrow is 8N and 16N is the most common ploidy stage found in humans. Conditions associated with large platelets have more megakaryocytes with lower ploidy (shifted left). These large platelets are called stress platelets like stress reticulocytes seen in anemias. Increased ploidy of megakaryocyte results in more cytoplasm and thus more platelet production from the cytoplasm. The stages of megakaryocyte can be differentiated on the basis of quantity and

characteristics of the cytoplasm, and the size, lobulations and chromatin pattern of the nucleus. A mature platelet is 2-4 micrometer in diameter. Volume is 7.06 ± 4.85 micro m3. Thickness is 0.9 ± 0.3 µm. The normal life span is 8-12 days.

In the stored blood life span is 1-2 days. Platelet turnover is 1.2-1.5 x 10^{11} /day. Once released from the marrow platelets are trapped in the marrow for 36 hours. 60-75% of the circulating platelets are in the blood. The remainder is in the spleen. Normal values for platelet numbers in peripheral blood vary with the method used for their estimation. Normal range is 1.5 lakh - 4.5 lakh/cumm.[16, 17]

Figure: 3 Megakaryopoiesis



FUNCTIONS OF PLATELETS

1.Haemostasis: - Immediate reaction following vascular injury is vasospasm. Next reaction is formation of platelet plug. Following endothelial injury, platelets come in contact with sub endothelial collagen, vWF and proteoglycans in the vessel wall.

They exhibit 3 reactions

a) Adhesion

b) Secretion and activation. The activated platelet changes its shape from disc shape to spherical increasing surface tension and forms pseudopodia and then release their granules.

c) Aggregation. The activated platelets stick to one another which is termed as platelet aggregation. Aggregation is also fostered by platelet activating factor. Primary platelet plug is formed which gets reinforced by fibrin to form stable platelet plug.

2. Growth factors that secreted by platelets causes vascular endothelial cells, vascular smooth muscle cells and fibroblasts to multiply and grow that helps repair damaged vascular walls.

3. They maintain the capillary integrity. This is evident by the fact that in thrombocytopenia due to any cause endothelium thins out with development of more fenestrations. Haemostasis is necessary to arrest blood flow. Once the bleeding stops, the damaged vessel are remodelled to restore normal blood flow.

The major components of the haemostatic system are:

1. Platelets

2. The coagulation and fibrinolytic factors and inhibitors

3. The vessel wall.[16, 17]

PLATELET PLUG FORMATION

Following vascular injury, platelets adhere to the endothelium by von Willebrand factor (vWF), through Gp Ib receptor. Platelet adhesion causes platelet activation, changing shape of the platelet from disc shape to spherical. Activated platelets then releases ADP and Thromboxane A2, that promotes aggregation and inhibits the normal anticoagulant property of endothelial cell. Platelet aggregation further causes recruitment of the platelets to the site of injury, leading to thrombus formation. After this secondary haemostasis takes place with the formation of fibrin and thus, the stable platelet plug.

Glycoprotein (Gp) IIb/IIIa is an important receptor for platelet aggregation and is the most abundant receptor present on the platelet surface. During platelet activation, inactive Gp IIb /IIIa receptor is converted into an active form, which helps binding of this complex to fibrinogen and vWF.

Activated platelets at the site of injury can rapidly form thrombus as each platelet surface has about 50,000 Gp IIb /III a fibrinogen binding sites. Gp IIb /III a has become an effective target for antiplatelet therapy as this receptor is important for platelet aggregation.[15]

Fibrin Clot Formation

Activation of coagulation cascade leads to the formation of fibrin. There are two pathways- extrinsic/tissue factor pathway and intrinsic/contact activation pathway.

Coagulation is initiated by the exposure of tissue factor (TF) and activation through the classic extrinsic pathway that proteolytically cleaves inactive proenzyme to activated thrombin which intern converts fibrinogen into fibrin.[14-16]

Figure: 4 Image of plug formation

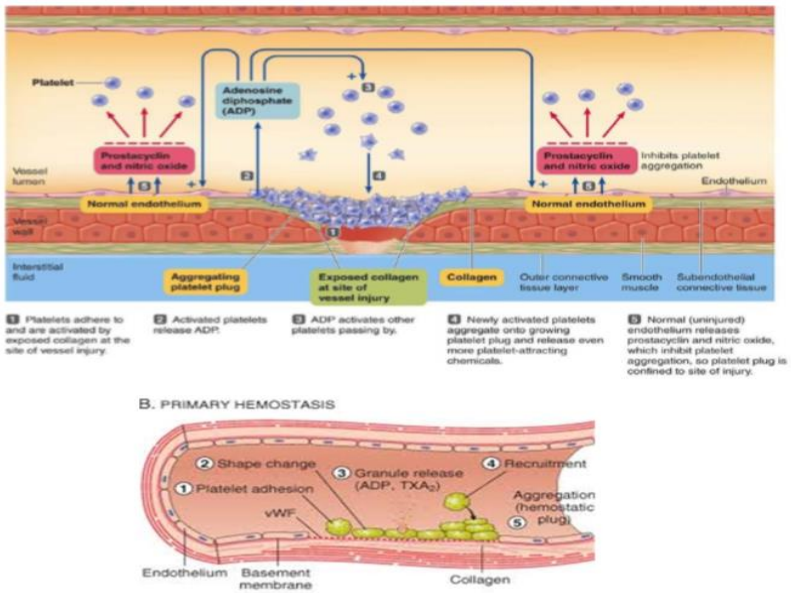
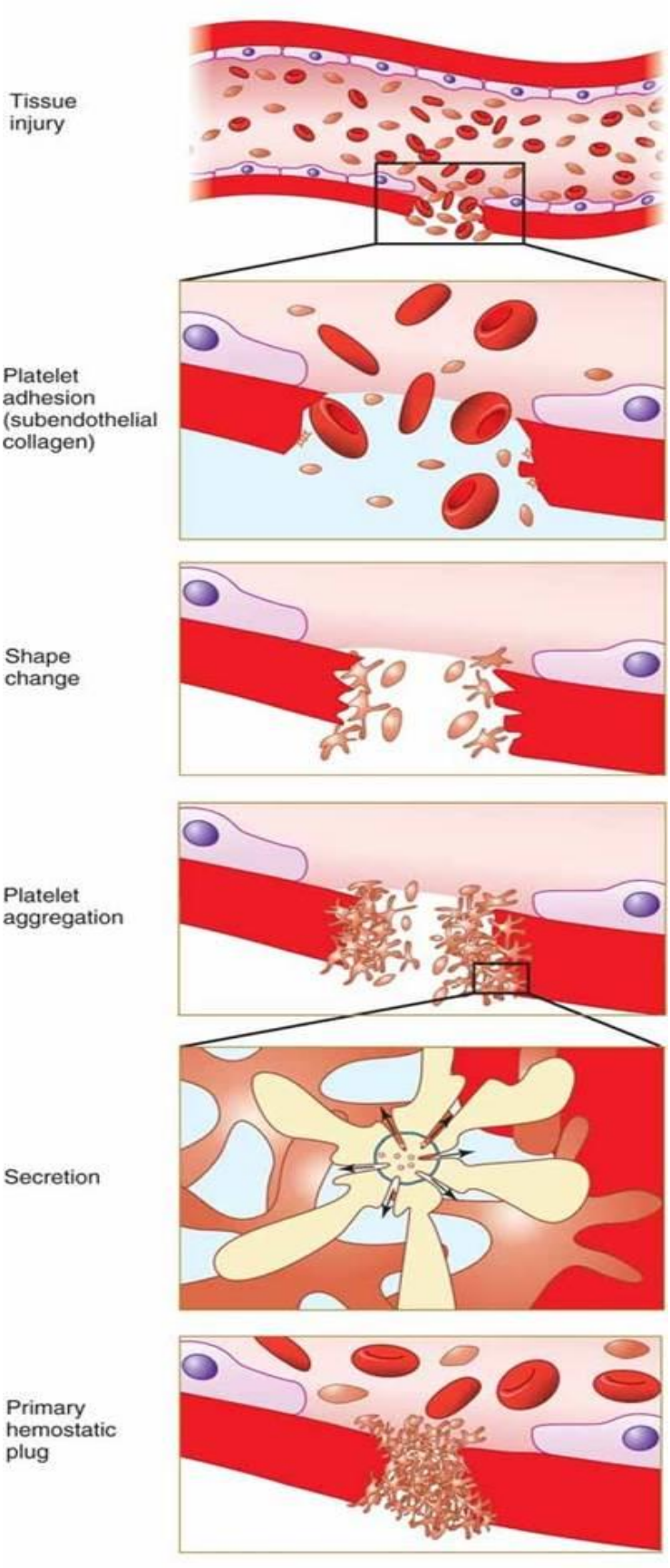


Figure: 5 Platelet activation:



HIISTORY OF THROMBOCYTOPENIA

In the year 1966, John Wright described that platelets are derived from megakaryocytes. Adelson E described platelets as sponges. In the year 1968, Behnke O described the electron microscope structure of platelet membrane. Penington DG ,Oslen TE, Fauser AA and Ebbe S, Yee T showed that thrombopoiesis is regulated by humoral mechanisms megakaryocytes respond by an increase in number, size, ploidy and increase in the rate of maturation whenever there is a decrease in the platelet count either due to immune destruction on increased removal.[5,6]

An estimated 15000000 megakaryocytes /kg body weight, averaging 12000 fL in volume, produce approximately 1000–1500 platelets each. Platelet counts normally range between 1.5 lakhs to 4.5 lakhs per micro litre and a count below 1.5 lakh is generally considered to constitute thrombocytopenia. Platelets were low in case of purpura and platelet count increases as the haemorrhage is ceased, Krauss (1883) and Denys (1887) stated that which was confirmed by Hayem in (1895). [12]

THROMBOCYTOPENIA

Thrombocytopenia may be due to any one or more than 4 causes:

1. Reduced platelet production

2. Increased platelet destruction

3. Artifactual Thrombocytopenia

4. Abnormal distribution or pooling of platelets within the body.

If the first platelet count is low, it should be confirmed by the second platelet count to label it as thrombocytopenia.[1]

Although bleeding tendency increases as platelet count decreases necessitating the need for a platelet transfusion. However, most of the patients with platelet count 20,000/cumm do not necessarily bleed. [1]

As the platelet count decreases, risk of bleeding increases.

<50,000-bleeds on clinical insult

<30,000-petechiae, menorrhagia and bruising

<20,000-spontaneous bleeding[1]

CAUSES OF THROMBOCYTOPENIA

DECREASED MARROW PRODUCTION

• Marrow failure- aplastic, hypoplastic anaemia's, drug effects

INCREASED DESTRUCTION OF CIRCULATING PLATELETS

Increased platelet destruction stimulates thrombopoiesis and thus leads to an increase in the number, size and rate of maturation of precursor megakaryocytes. It can occur both intracorpuscular as well as extracorpuscular.

Platelet destruction is most often the result of extracorpuscular destruction and among this, immunologic cause is most common. Intracorpuscular destruction is rare and is seen in some forms of hereditary thrombocytopenia such as Wiskott Aldrich syndrome.

Mechanisms of platelet destruction

1. Immune mediated

a) Autoantibody-mediated platelet destruction via reticuloendothelial system (RES) Example: ITP Secondary - lymphoproliferative disease, collagen vascular disease, infections such as infectious mononucleosis and HIV

b) Alloantibody – mediated platelet destruction via RES Example: Neonatal alloimmune thrombocytopenia; post transfusion purpura.[18]

c) Antibodies against microbial antigens adsorbed onto platelets Example: Malaria associated thrombocytopenia.[19]

d) Drug-dependent, antibody-mediated platelet destruction via RES

Example: Drug-induced immune thrombocytopenic purpura (quinine, quinidine, sulfa drugs, vancomycin, etc).[20]

e) Platelet activation by binding of IgG Fc of drug-dependent IgG to platelet Fc-R IIa receptors Example: Heparin induced thrombocytopenia.[21]

2. Non-immune mediated

a) Platelet activation via thrombin or inflammatory cytokines. Example: Disseminated Intravascular Coagulation (DIC), septicaemia and other systemic inflammatory response syndromes (adult respiratory distress syndrome, fat embolism and pancreatitis).

b) Platelet destruction via ingestion by macrophages (haemophagocytosis. Example: Infections and malignant lymphoproliferative disorders.[22]

c) Platelet destruction via platelet-activating proteinase

Example: Haemolytic uremic syndrome and Thrombotic thrombocytopenic purpura.[22-24]

DECREASED PLATELET PRODUCTION

Multiple cell lines get affected, as a result thrombocytopenia is accompanied by varying degrees of anaemia and leukopenia, although thrombocytopenia may be the initial manifestation. Most common cause of decreased production is marrow aplasia, marrow fibrosis, infiltration with malignant cells, ineffective thrombopoiesis or disorders of

thrombopoietic control. Defect is confirmed by Bone Marrow aspiration or Bone Marrow biopsy.[25]

ABNORMAL PLATELET POOLING

1. Disorders of spleen (Neoplasm, congestive, infiltrative)

2. Hypothermia.

3. Dilution of platelets with massive transfusions.

ARTIFACTUAL THROMBOCYTOPENIA

Artifactual thrombocytopenia refers to falsely low platelet counts. It should be taken into consideration in patients with low platelets but no bleeding manifestation.

Causes of artifactual thrombocytopenia are:

Giant platelets up to 10% of normal platelets are giant platelets (larger than usual sites) if > 20% of platelets are giant, the differential diagnosis of Bernard-Soulier syndrome– BSS , ITP, lympho- and myeloproliferative disorders, reticulocytosis, DIC, SLE, gray platelet syndrome, May-Hegglin anomaly, Montreal platelet syndrome and TTP should be considered.[26]

Platelet satellitism:

Platelet adherence around polymorphonuclear neutrophils is termed as platelet satellitism. This is observed in blood treated with EDTA at room temperature. Heparin and citrate do not produce rosetting. The proposed mechanisms include immunologic bonding through EDTA dependent antiplatelet and anti-neutrophil IgG autoantibodies directed against the glycoprotein IIb/IIIa complex and Fc γ receptors of platelets and neutrophils. Phagocytosis of platelets by polymorphonuclear leukocytes and monocytes is often seen. Platelet satellitism ia a cause of pseudo thrombocytopenia.[26, 27]

THROMBOCYTOPENIA ASSOCIATED WITH INFECTION:

Infection is a common cause of thrombocytopenia, occurring in approximately 50–75% of patients with bacteraemia or fungemia, septic shock and DIC.[28] In bacteraemia, the thrombocytopenia is generally mild to moderate in severity, and usually not accompanied by significant coagulation abnormalities or bleeding. In septicaemia without DIC thrombocytopenia is caused due to chemokine-induced macrophage ingestion of platelets (haemophagocytosis) and direct activation of platelets by endogenous mediators of inflammation (e.g., platelet activating factor)[29] or certain microbial products.[30] Prompt recognition and treatment of the infection is most important, as platelet count normalities with the resolution of the infection. Prophylactic platelet transfusions are not required unless the platelet count falls to $<10 \times 10^9$/lakh, or unless comorbid clinical features increase the

likelihood of serious bleeding (e.g. concomitant coagulopathy, an invasive procedure, uremic platelet dysfunction). Immune mediated platelet destruction, impaired platelet production secondary to HIV infection of megakaryocytes, drug- induced myelosuppression (commonly, zidovudine, ganciclovir, and trimethoprim /sulfamexthoxazole), HIV- associated thrombotic microangiopathy, hypersplenism, and marrow infiltration by tumor or opportunistic infections are potential causes of thrombocytopenia in HIV patients.[31] Viral causes include- Dengue, HIV, CMV, HSV, Parvo-B19, Hanta virus etc.[32] Viruses produce thrombocytopenia by impairment of platelet production as a result of viral invasion of megakaryocytes, toxic effects of viral protein on progenitor cells, virus induced haemophagocytosis, viral antigen-antibody complex mediated.[33]

Bacterial causes include- Gram positive and gram negative septicaemia, leptospirosis, miliary tuberculosis, typhoid, mycoplasma pneumonia.[34]

Septicaemia resulting from both gram negative and gram positive bacteria is the commonest cause of thrombocytopenia. It may be caused by disseminated intravascular coagulation (DIC) and the diagnosis of DIC may be apparent when coagulation studies are performed.[35]

Disseminated intravascular coagulation (DIC) is a disorder involving consumption of platelets and pro-coagulants with the formation of fibrin, resulting in intravascular coagulation and haemorrhage. It is an acquired syndrome characterized by the intravascular activation of coagulation and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction.[36] DIC is a complication of other illnesses rather than a

primary event and present in almost 1% of all the hospitalized patients.[37] DIC is associated with various clinical conditions and is secondary to an underlying disorder, results from activation of systemic inflammation. DIC leads to depletion of clotting factors, intravascular coagulation, and end-organ damage. DIC is frequently associated with severe sepsis and septic shock.[38, 39]

Platelets adherence to damaged vascular surfaces also accounts for thrombocytopenia in certain bacterial infections, such as meningococcemia. Endotoxin, exotoxin, platelet activating factor may damage platelets, resulting in an increased clearance.

Protozoal causes: Thrombocytopenia occurs in over 80% of patients with malaria and human platelets have been demonstrated to contain plasmodia species.[32] It was immune mediated destruction with elevated platelet activated immunoglobulin. It was demonstrated that ultra-structural changes in platelets and the level of parasitemia was the cause for thrombocytopenia. Antibodies against microbial antigens adsorbed onto platelets.

Causes of altered platelet indices

Decreased platelet indices seen in Alzheimer's disease, vascular dementia, pulmonary arterial hypertension and osteoporosis.

Increased platelet indices are seen in myocardial infarction, unstable angina and vascular complications of diabetes.[40]

Moreau et al studied the role of platelet parameters in diagnosing various clinical conditions, concluded that once platelets get activated, there is change of shape and platelet swelling leading to increased MPV and PDW. Correlation between platelet count and MPV was done, inverse relation was found in cases of anemia. On platelet activation, MPV and PDW increases and MPV is increased in ITP and lower in AML.[41]

According to Sharma et al, platelet activation is indicated by MPV and it indirectly tells about the bone marrow activity. But no significant correlation was found between MPV and severity of the disease, thus MPV does not serves as a prognostic parameter in dengue fever.[11]

According to a study done on -Thrombocytopenia in Malaria: Can platelet counts differentiate malaria from other infections. Journal of the College of Physicians and Surgeons, concluded that platelet count is very specific for the diagnosis of vivax malaria .[42]

Higher the platelet count lower is the bleeding tendency. Increased IPF and large platelets are associated with increase bleeding tendency. Unstimulated platelets with increased number of P selectins on their surface is associated with increased bleeding tendancy.[43]

MPV indicates platelet activation, platelet aggregation, glycoprotein IIb/IIIa expression and production of thrombogenic factors. Platelet size is independent of age of the platelet and its regulation is multifactorial. Increased MPV seen in patients with cardiovascular risk factors-smoking, diabetes, obesity, hypertension and hyperlipidemia.[44]

Platelet distribution width (PDW) is the relative width of platelet distribution in volume index of the platelet heterogenicity. PDW are elevated in patients with COPD with pulmonary embolism and correlates with bilateral thrombus.[13]

Megakaryopoiesis is regulated by thrombopoietin. For patients having Hepatocellular carcinoma with chronic liver disease, as well as patients with infective endocarditis, platelet size is a significant predictor. MPV ranges from 7-11 fL. Increased MPV is seen in coronary artery diorders.[14]

MPV is high in thrombocytopenia due to increased platelet production as well thrombocytopenia due to increased platelet destruction. MPV values also tend to be high when using EDTA as an anticoagulant due to swelling of the platelets. On platelet destruction, cytokines especially IL-6 causes release of more reactive and larger platelets.[15]

Once the platelets gets activated, shape of the platelets changes from discoid to spherical and acquires more surface area and leads to pseudopodia formation. PDW varies with the increased number and size of pseudopodia. On platelet activation, both MPV and PDW increases, PDW being more specific indicator of platelet activation but no change in platelet count. Large platelets can be seen in various conditions- Haemorrhage and myeloproliferative conditions. In a study done on the relationship between platelet indices with testicular artery blood flow and fertility, the role of platelet activation was investigated. They found there is no statistical correlation between platelet and platelet indices and testicular artery blood flow, although platelet activation plays an important role in atherosclerosis. Large platelets are more active than small platelets both enzymatically as well as metabolically and express

more number of procoagulatory proteins like Glycoprotein IIIa and P-selectin. Megakaryocyte ploidy has a relation with platelet volume, though the mechanism is not clear. P selectin, is a glycoprotein belongs to a member of C type lectin family, on stimulation, they move from alpha granules and weibel palade bodies of endothelial cells to the cell surface.

P selectin plays an important role in –

1. Rolling of platelets on activated endothelial cells.
2. Interaction of activated platelets with neutrophils and monocytes.
3. Platelet aggregation.

Platelet aggregates are formed by the interaction of GP IIb/IIIa and fibrinogen interaction. P selectin stabilises these platelet aggregates by binding on the platelet surface through lectin domain and thus, allows formation of larger platelet aggregates.

In Glanzman's thrombocytopenia, there is no platelet aggregation in spite of presence of P selectin, suggesting that initial interaction of GP II b /III a and fibrinogen is important for the function of P selectin. P selectin helps in estimating the size and stability of platelet aggregates. Anti P-selectin inhibits P selectin binding to its ligand. [15-17]

According to a study done on mean platelet volume may represent predictive parameter for overall vascular mortality and ischemic heart disease, they concluded that when platelet gets

activated, they undergoes morphological changes both in the form of spherical shape and pseudopodia formation, thus affecting platelet distribution width (PDW) [45]

Out of three platelet indices, Platelet distribution width is definite marker of platelet activation as there is no change during simple platelet swelling. Platelet hyper activation promotes thrombus formation .[45, 46]

Platelet indices are simple, inexpensive, non invasive and raised values not only predict platelet activation but also suggest a destructive etiologies with compensatory increased marrow production and low platelet indices suggest a hypoproductive marrow state.[47]

The hypothesis for the present study was that enzymatically active platelets are larger in size and hence such cases have raised platelet indices. Thus patients with lower platelet count and low platelet indices are more likely to bleed than patients with similar platelet count but raised platelet indices (MPV, PDW and P-LCR).

MATERIALS AND METHODS:

SOURCE OF DATA:

All cases of thrombocytopenic patients admitted to R.L Jalappa hospital attached to Sri Devaraj Urs Medical College from December 2014 to July 2016 were included in the study.

STUDY DESIGN: Short term cross sectional observational study

DURATION OF STUDY - DECEMBER 2014 to JULY 2016 (1 YEAR AND 8 MONTHS).

METHOD OF COLLECTION OF DATA:

2 mL of venous blood sample was collected in 2 vacutainers (one with EDTA anti-coagulant and the other citrate anticoagulant) – one for CBC and the other for PT, aPTT and INR respectively.

CBC was done by automated Hematology Analyzer **ALERE H 560**, smears prepared and slides stained.

Principle: Volumetric impedance (the Coulter method) is used to determine the cellular concentrations and volume distributions of WBC, RBC and Platelets. Photometric measurement of light absorbance is used to determine hemoglobin concentration.

PT, aPTT and INR was done using STAGO START 4 COAGULATION ANALYSER to exclude the cases with abnormal coagulation profile. Blood samples were collected in a vacutainer with 3.2% sodium citrate as anticoagulant in the ratio of 1: 9.

Principle for PT: consists of the use of calcium thromboplastin to measure the clotting time of the patient‘s plasma and to compare it with that of a normal standard. The test measures as a whole, activity of the coagulation factor II (prothrombin), factor V (proaccelerin) factor VII (proconvertin), factor X (Stuart factor) and factor I (fibrinogen).

Normal range=12-16secs.

Principle for APTT: the APTT involves the recalcification of plasma in the presence of a standardized amount of cephalin (platelet substitute) and a factor XII activator (kaolin). The APTT explores the intrinsic coagulation pathway.

Normal range= 26-34secs.

Then samples were grouped in 2 categories:

Group: 1 Platelet count <1.5lakh/cumm without bleeding

Group: 2 Platelet count <1.5lakh/cumm with bleeding.

In these 2 groups, platelet indices to be compared according to the following platelet count.

<20,000/cumm

20,000-50,000/cumm

>50,000/cumm

For the present study,

Normal platelet count = 1.5 lakh/cumm –4.5 lakh/cumm[1]

Normal MPV range = 8-12 fL [1]

Normal PDW range = 9-15fL [1]

Normal Plateletcrit = 20 – 36%

Low platelet count (Thrombocytopenia) i.e. <1.5lakh/cumm

Low MPV i.e. <8fL and high MPV >12fL

Low PDW i.e. <9fL and high PDW >15fL

SAMPLE SIZE:

Sample size was calculated according to the pilot study.

It includes all the thrombocytopenic patients with platelet count <1.5L/cu mm

$$n=4pq/d^2$$

p: prevalence of bleeding in thrombocytopenic patients=5%

q=100-p =95%

d:absolute error=5%

n=4x5x95/25 =76%

+/- 10% Non-responsive

n= 76+7 =83 cases of thrombocytopenia with platelet count<1.5L/cu mm with bleeding and similar number of cases i.e. 83 cases were included without bleeding for comparison of platelet indices in thrombocytopenic patients with bleeding and without bleeding.

Thus, total number of cases = 166 thrombocytopenic cases, 83 cases with bleeding and 83 cases without bleeding.

190 cases were been collected during the period of 1 years and 8 months and then, platelet indices and bleeding tendency were been studied in all patients with platelet count <1.5lakhs/cu mm

CLINICAL ASSESSMENT OF BLEEDING TENDENCY:

Examination of the following features based on WHO criteria.

Oropharyngeal bleeding

Conjuctival bleed

Petechiae of oral mucosa

Petechiae of skin

Epistaxis

Hematomas

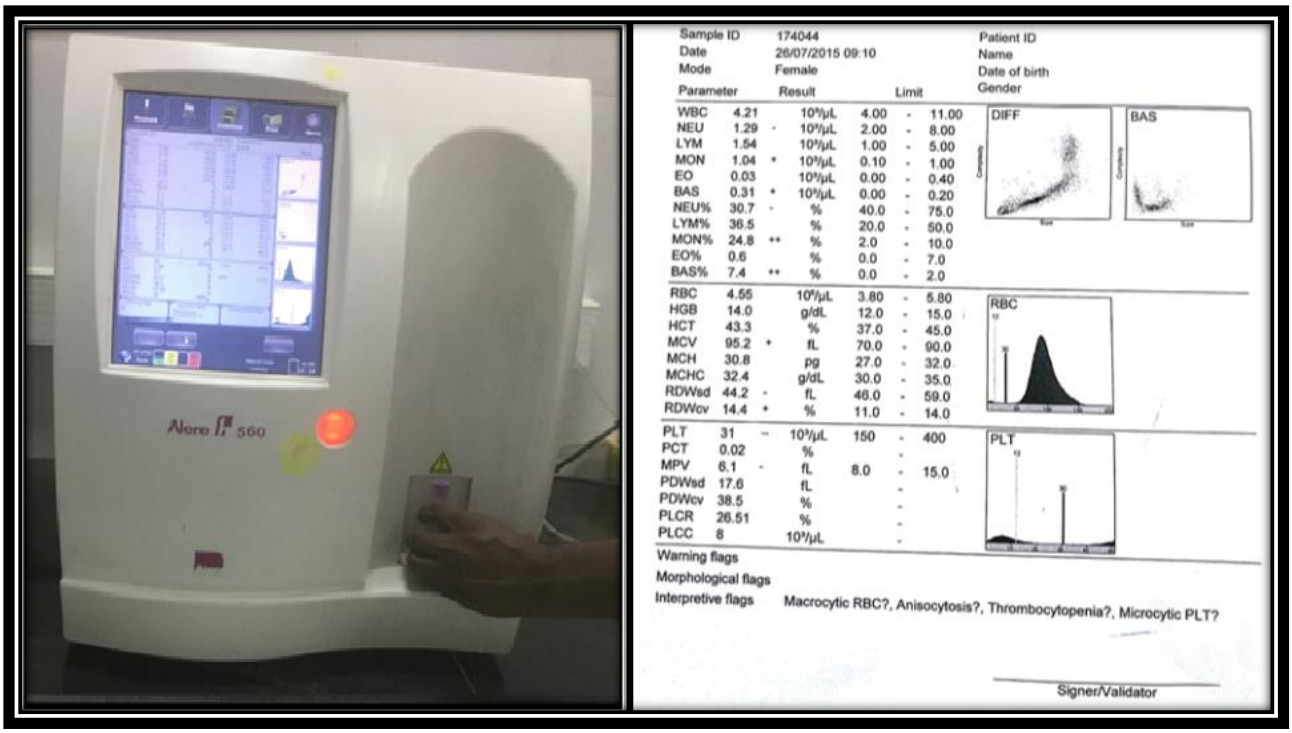
Positive stool occult blood

Microscopic in urine RBCs - Hematuria

Haemoptysis

Melena

Figure: 6 Haematological analyser ALERE H 56



INCLUSION CRITERIA:

All adult patients >18 years with platelet count below 1.5lakh/cumm in the following conditions:

1) Case of febrile and immune thrombocytopenia including Dengue/malaria/viral fever
2) Obstetric/ gynaecalogical cases
3) Leukemia.

EXCLUSION CRITERIA:

1) Patients with abnormal coagulation profile.
2) Vasculitis

STATISTICAL ANALYSIS:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square was used as test of significance. Continuous data was represented as mean and SD. Independent t test was used as test of significance to identify the mean difference between two groups and ANOVA (Analysis of Variance) was the test of significance to identify the mean difference between more than two groups. P value <0.05 was considered as statistically significant. To verify the association between all platelet indices, Pearson's correlation was applied and (r) value 1 was taken as statistically significant.

Correlation Coefficient (r)	Interpretation
0-0.3	Positive weak correlation
0.3-0.6	Positive moderate correlation
0.6-1.0	Positive strong correlation
0 to (-0.3)	Negative weak correlation
(-0.3) to (-0.6)	Negative moderate correlation
(-0.6) to –(1)	Negative strong correlation.

RESULTS:

190 cases of thrombocytopenia were studied, 95 cases with bleeding and 95 cases without bleeding during the period of December 2014 to July 2016 in the Dept. of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar.

The following parameters are discussed below:

Age and sex distribution

Distribution of platelet count and etiology for thrombocytopenia

Distribution of symptoms in thrombocytopenic patients

Distribution of platelet count, bleeding and etiology for thrombocytopenia

Mean platelet volume with respect to bleeding

Platelet indices with respect to platelet count

Distribution of anemia and platelet count

Comparison of bleeding manifestation with platelet indices

Correlation between platelet count, MPV, PDW and plateletcrit among subjects with bleeding and without bleeding

Table 1: Age distribution of subjects with bleeding

		Gender			
		Female		Male	
		Count	%	Count	%
Age	<20 years	10	9.1%	10	12.5%
	21 to 30 years	45	40.9%	18	22.5%
	31 to 40 years	26	23.6%	20	25.0%
	41 to 50 years	19	17.3%	14	17.5%
	51 to 60 years	6	5.5%	9	11.2%
	>60 years	4	3.6%	9	11.2%
Total		110		80	

As shown in **table:1** , most of the females patients (40%) were in the age group of 21-30 years and least were in the age group of >60 years while, most of the males (25%) were in the age group of 31-40 years and least among >40 years. There was female predominance in all age group except after age group of >50years. Female to male ratio was 1.375:1

Table: 2 Distribution of mean age, platelet count with bleeding and without bleeding

		Bleeding					
		Present			Absent		
		No. of cases	Mean Age(years)	SD	No. of cases	Mean Age(years)	SD
Platelet	< 20000/cumm	20	37.7	13.4	10	39.6	15.0
	20000 to 50000/cumm	44	33.8	12.3	36	37.5	16.3
	> 50000/cumm	31	34.0	16.2	49	39.6	16.0
P value		0.556			0.825		

As shown in **table: 2 and figure:8**, there was no significant difference in age distribution between subjects with and without bleeding with respect to platelet count.

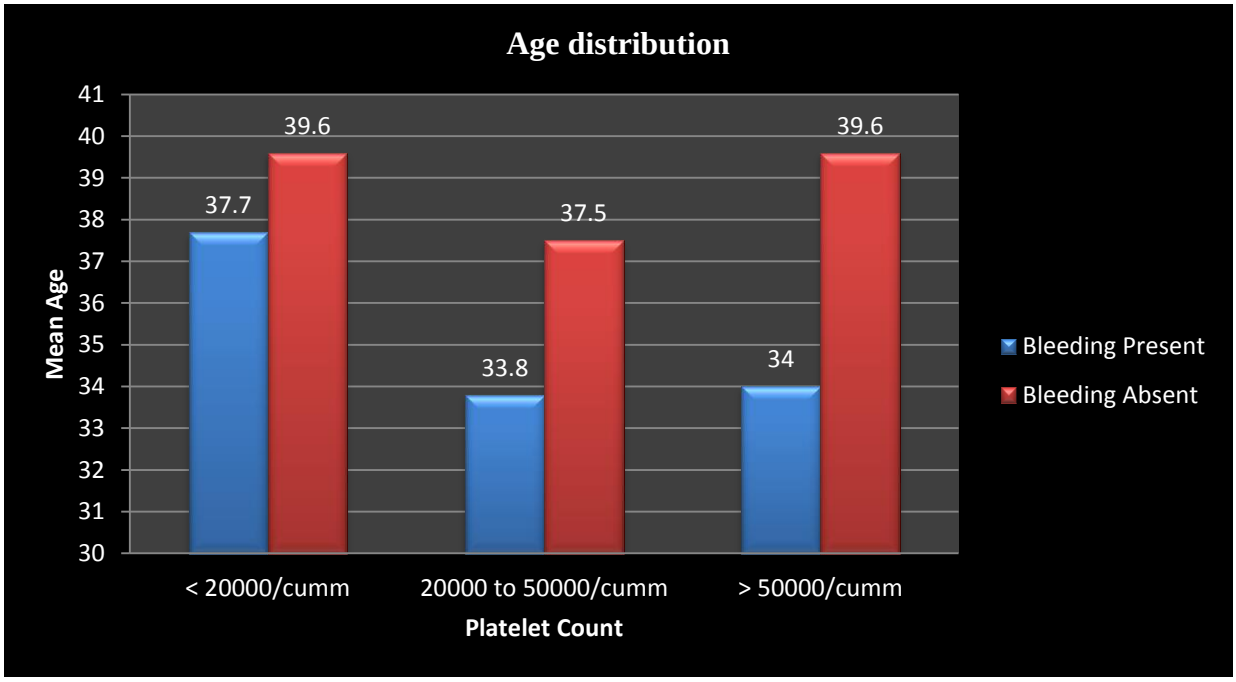


Figure 8: Distribution of Mean age of subjects with respect to platelet count

Table 3: Distribution of Gender and bleeding

		Bleeding			
		Present		Absent	
		No. of cases	%	No. of cases	%
Gender	Female	51	53.7%	59	62.1%
	Male	44	46.3%	36	37.9%
	Total	95	100.0%	95	100.0%

$\chi 2 = 1.382$, df = 1, p = 0.240

As shown in **table: 3** and **figure: 9,** majority of subjects in the study were females in the study. There was no significant association between gender and platelet count and bleeding manifestations.

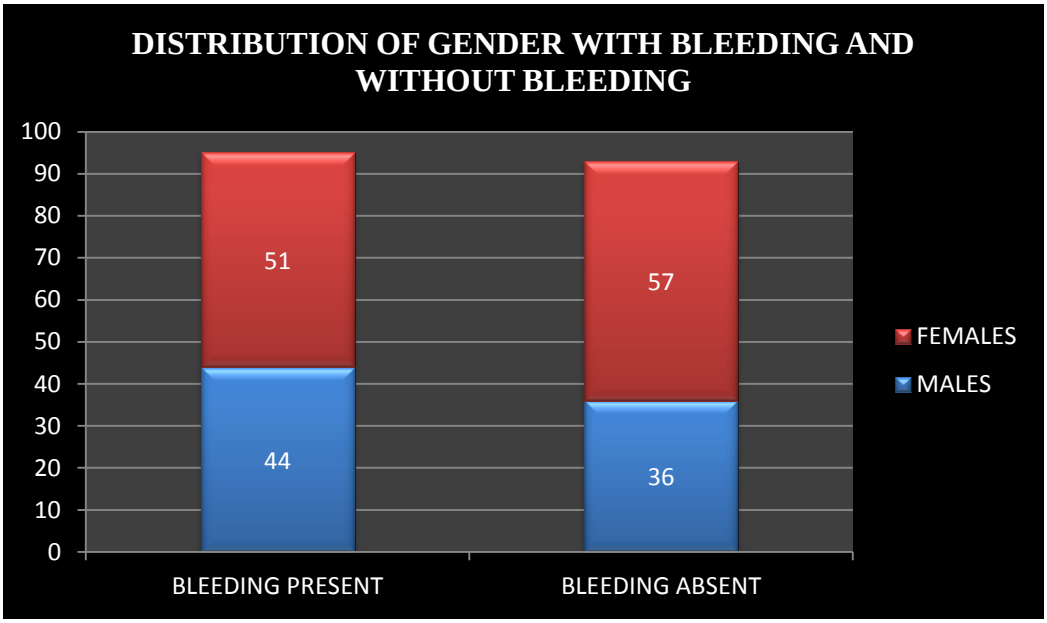
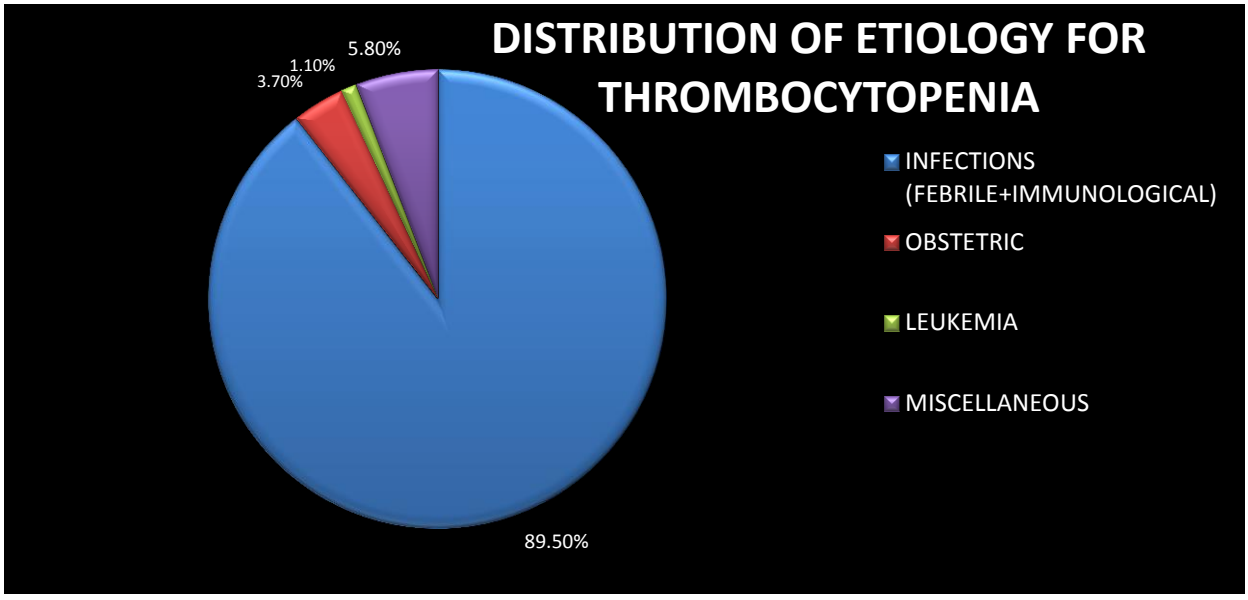


Figure 9: Distribution of gender and bleeding.

Table 4: Distribution of Etiology for thrombocytopenia

		Count	%
Diagnosis	Leukemia	2	1.1%
	Infectious-(Febrile + Immunological)	170	89.5%
	Miscellaneous	11	5.8%
	Obstetric	7	3.7%
	Total	190	100.0%



$\chi 2 = 3.983$, df = 6, p = 0.679

Figure: 10 Distribution of platelet count and etiology for thrombocytopenia

As shown in **table:** 4 and **figure:10**, majority of subjects with thrombocytopenia were found to have acute febrile illness i.e. 89.5% cases.

Table 5: Distribution of symptoms

Symptoms	≤20 yrs.	21-30yrs	31-40yrs	41-50yrs	51-60yrs	>60yrs	Total	Percentage (%)
Hematemesis	0	1	2	1	0	0	4	4.21
Gum bleed	4	6	5	9	3	1	28	29.47
Hematuria	3	2	6	5	1	0	16	16.84
Melena	3	2	4	2	1	2	14	14.74
Petechiae oral cavity	4	4	6	2	3	0	20	21.05
Petechiae skin	1	2	2	2	1	0	8	8.42
Conjuctival bleed	0	1	2	2	0	1	9	9.47
Epistaxis	2	2	2	3	0	0	17	17.89
I/V cannula	1	0	2	0	0	0	3	4.21

As shown in **table: 5**, most frequent bleeding manifestation was bleeding gums (29.47%) followed by petechiae oral cavity (21.05%) and third common were epixtaxis (17.89%)

Table 6: Association between Platelet count and Etiology for thrombocytopenia

					Platelet					
					<20000/cumm		20000 to 50000/cumm		>50000/cumm	
					Count	%	Count	%	Count	%
Diagnosis	Infectious	28	93.3%	73	91.2%		69	86.2%		
	Obstetric	1	3.3%	2	2.5%		4	5.0%		
	Leukemia	0	0.0%	0	0.0%		2	2.5%		
	Miscellaneous	1	3.3%	5	6.2%		5	6.2%		

$\chi 2 = 3.983$, df = 6, p = 0.679

As shown in **table: 6** and **figure:11,** majority of subjects with thrombocytopenia were diagnosed to have acute febrile illness.

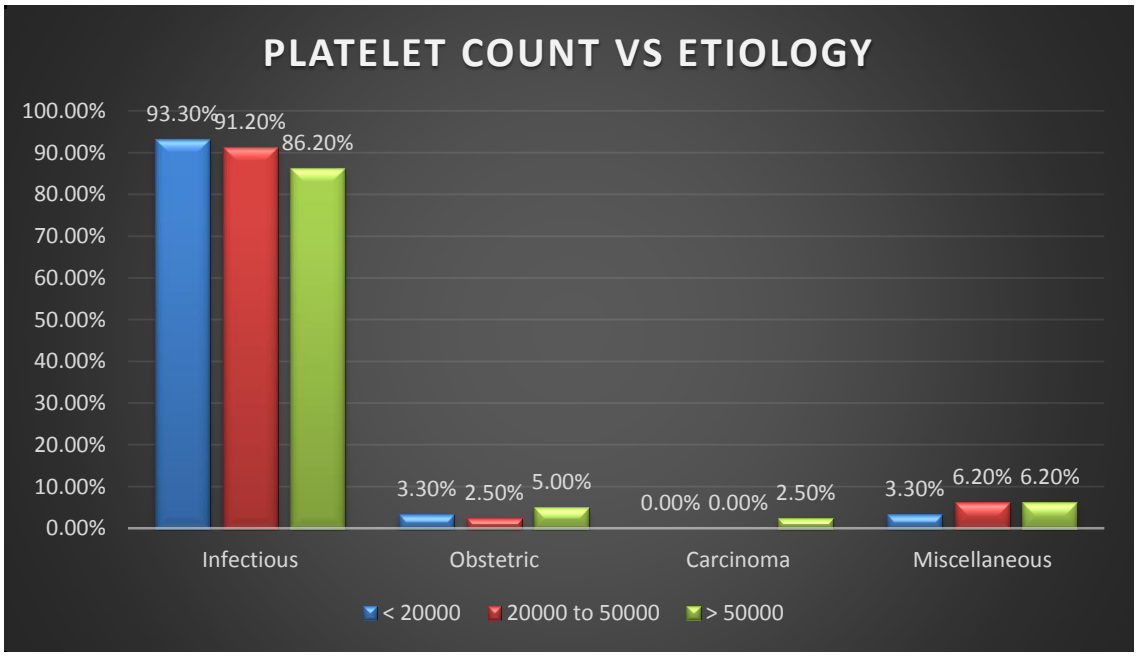


Figure 11: Distribution between Platelet count and etiology for thrombocytopenia.

Table 7: Distribution of cases with bleeding and without bleeding.

		Platelet							
		< 20000/cumm		20000 to 50000/cumm		> 50000/cumm		Total	
		Count	%	Count	%	Count	%	Count	%
Bleeding	Absent	10	33.3%	36	45.0%	49	61.2%	95	50.0%
	Present	20	66.7%	44	55.0%	31	38.8%	95	50.0%
	Total	30	100.0%	80	100.0%	80	100.0%	190	100.0%

$\chi 2 = 8.18$, df = 2, p = 0.017*

As shown in **table:7 and figure:12,** it was observed that 66.7% of subjects with platelet count < 20,000/cumm, 55.0% with platelet count 20,000 to 50,000/cumm and 38.8% of subjects with > 50,000/cumm had bleeding manifestations. Hence bleeding was more common with lower platelet count significantly.

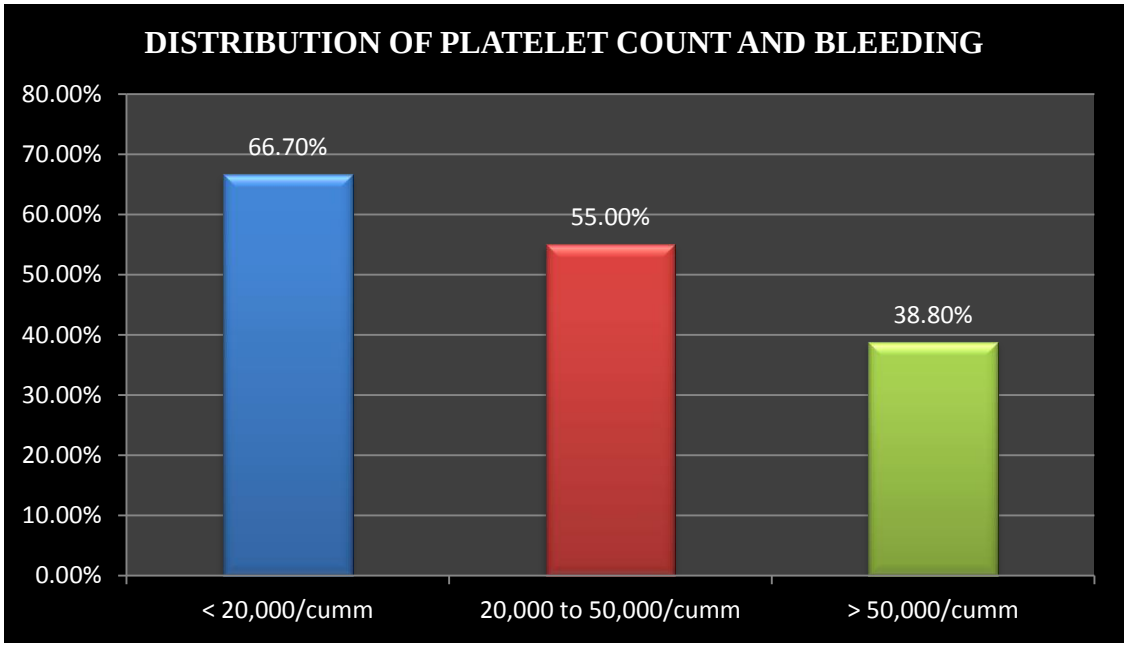


Figure 12 Distribution of platelet count and bleeding manifestation

Table 8: Distribution of Mean platelet count with respect to bleeding

		Platelet Count		
		Count	Mean(cumm)	SD
Bleeding	Present	95	44526.3	30242.2
	Absent	95	66042.1	40789.3
P value		190	<0.001*	

As shown in **table: 8,** among subjects who presented with bleeding mean platelet count was 44526.3 ± 30242.2/cumm and among without bleeding subjects was 66,042.1± 40789.3/cumm. This mean difference in platelet count was statistically significant.

Table 9: Distribution of MPV (Mean Platelet volume) and platelet count

				Platelet count						P value
				< 20000/ cumm		20000 to 50000/cumm		> 50000/ cumm		
				Count	%	Count	%	Count	%	
Bleeding	Present	MPV	Low	20	100.0%	43	97.7%	29	93.5%	0.394
			Normal	0	0.0%	1	2.3%	2	6.5%	
			High	0	0.0%	0	0.0%	0	0.0%	
	Absent	MPV	Low	3	30.0%	26	72.2%	20	40.8%	0.013*
			Normal	6	60.0%	6	16.7%	25	51.0%	
			High	1	10.0%	4	11.1%	4	8.2%	

As shown in **table: 9** and **figure:13 &14,** in subjects with bleeding, there was no significant association between platelet count and MPV grading.

Whereas in subjects without bleeding, there was significant association between platelet count and MPV grading. Among subjects with platelet count between 20,000 to 50,000, majority of them had low MPV, among subjects with platelet count <20000, 60% of them had normal MPV and among subjects with platelet count >50,000, 51.0% of them had normal MPV.

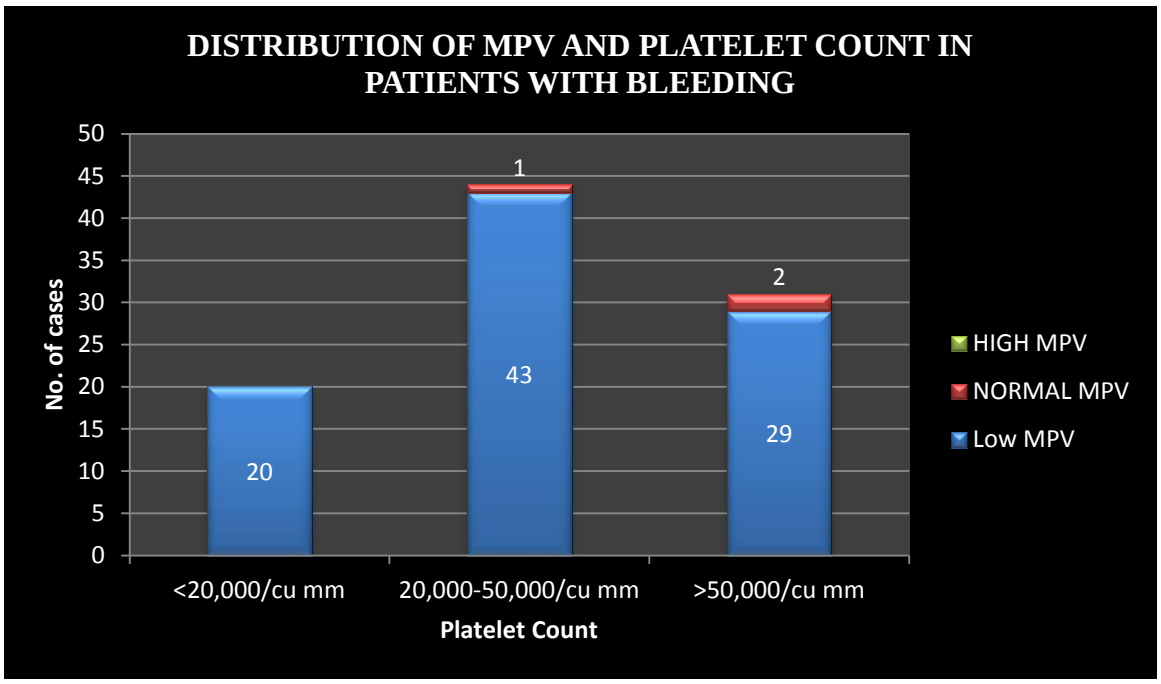


Figure 13: Distribution of MPV (Mean Platelet volume) and platelet count among subjects with bleeding

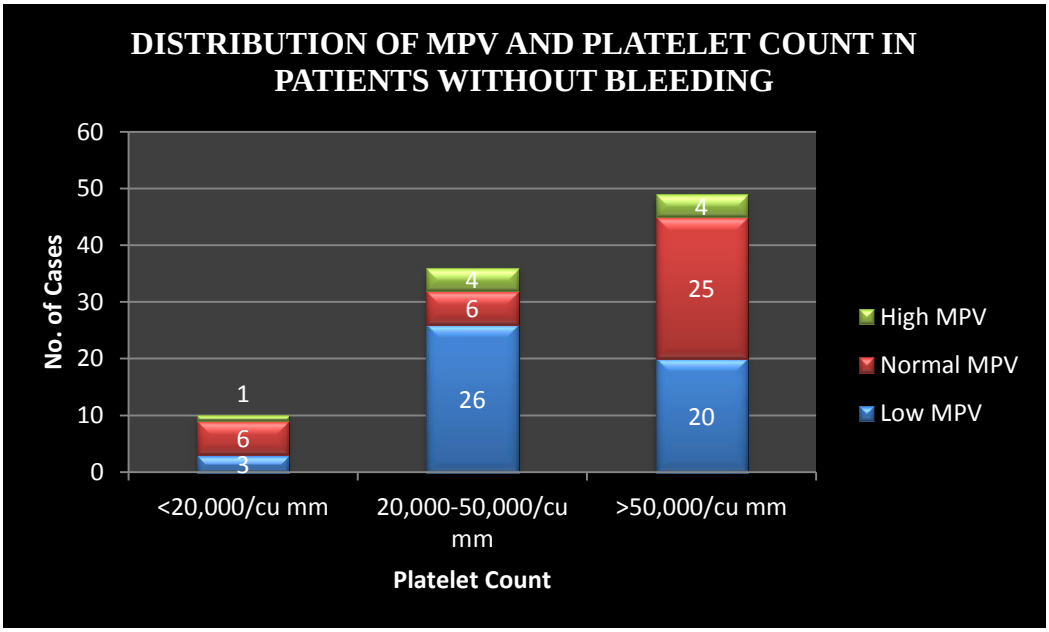


Figure 14: Distribution between MPV (Mean Platelet volume) and platelet count among subjects without bleeding

Table 10: Distribution of platelet indices with respect to platelet count

		MPV		PDW		PLCR		PLATELET CRIB	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Platelet count	<20000/cumm	6.2	2.1	12.1	9.5	21.4	7.2	20.8	7.2
	20,000 to 50000/cumm	6.2	1.6	18.9	4.9	25.8	6.6	24.7	7.0
	>50000/cumm	7.3	1.5	19.2	4.6	27.7	8.5	27.6	6.9
P value		<0.001*		<0.001*		0.001*		<0.001*	

As shown in **table: 10 and figure:15,** among subjects with platelet count <20,000/cumm, mean MPV was 6.2 ± 2.1, among 20,000 to 50000/cumm platelet count subjects mean MPV was 6.2 ± 1.6fL and among >50,000/cumm platelet count subjects mean MPV was 7.3 ± 1.5fL. Hence with decrease in platelet count there was also decrease in MPV significantly.

Among subjects with platelet count <20,000/cumm, mean PDW was 6.3 ± 9.5fL, among 20,000 to 50000/cumm platelet count subjects mean PDW was 18.9 ± 4.9fL and among >50,000/cumm platelet count subjects mean PDW was 19.2 ± 4.6fL. Hence with decrease in platelet count there was also decrease in PDW significantly.

Among subjects with platelet count <20,000/cumm, Mean plateletcrit was 20.8 ± 7.2%, among 20,000 to 50,000/cumm platelet count subjects mean plateletcrit was 24.7 ± 7.0% and among >50,000/cumm platelet count subjects mean PDW was 27.6 ± 6.9%. Hence with decrease in platelet count there was also decrease in plateletcrit significantly.

Among subjects with platelet count <20000/cumm, Mean PLCR was 21.4 ± 7.2%, among 21000 to 50000/cumm platelet count subjects mean PLCR was 25.8 ± 6.6% and among >50000/cumm platelet count subjects mean PLCR was 27.7 ± 8.5%. Hence with decrease in platelet count there was also decrease in PLCR significantly.

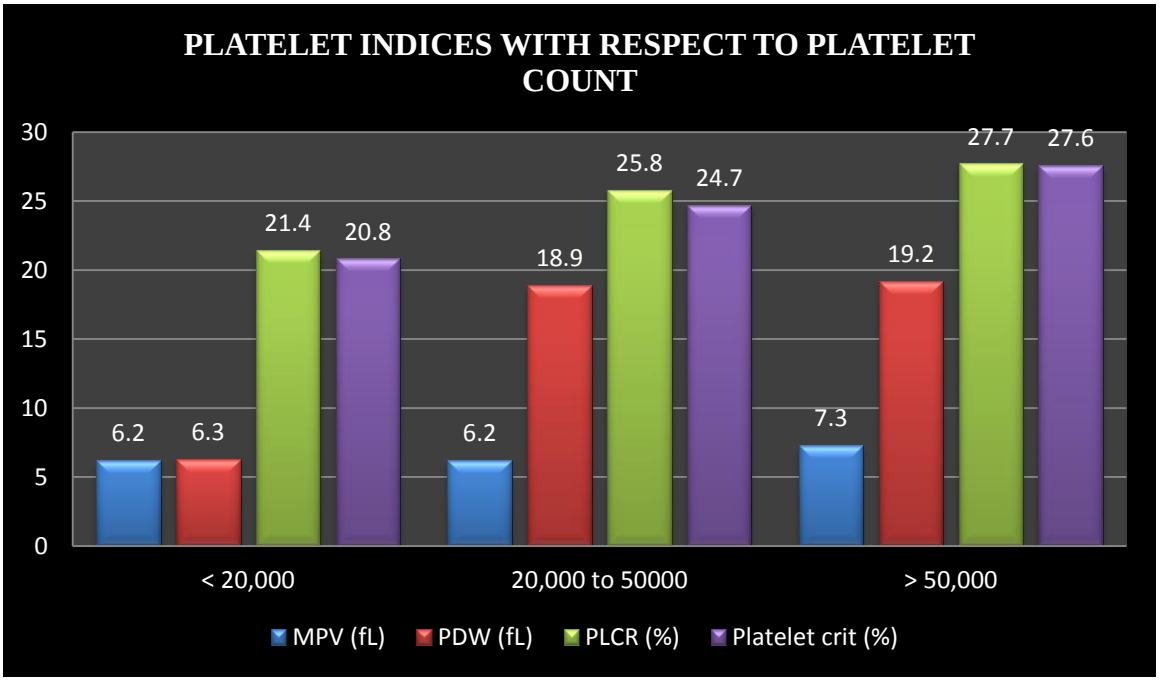


Figure 15: Platelet indices with respect to platelet count

Table 11: Distribution of Normocytic anemia and platelet count

		Platelet						Total
		< 20,000/cumm		20,000 to 50000/cumm		> 50,000/cumm		
		Count	%	Count	%	Count	%	
Anemia	Absent	19	63.3%	57	71.2%	40	50.0%	116(61%)
	Present	11	36.7%	23	28.7%	40	50.0%	74(39%)
Total		30		80		80		190

χ 2 = 7.674, df = 2, p = 0.022*

As shown in **table: 11 and figure:16,** significant association was observed between normocytic anemia and platelet count. In subjects with platelet count <20,000 36.7% had normocytic anemia, among subjects with platelet count 20,000 to 50,000 28.7% had normocytic anemia and among > 50000, 50% had anemia.

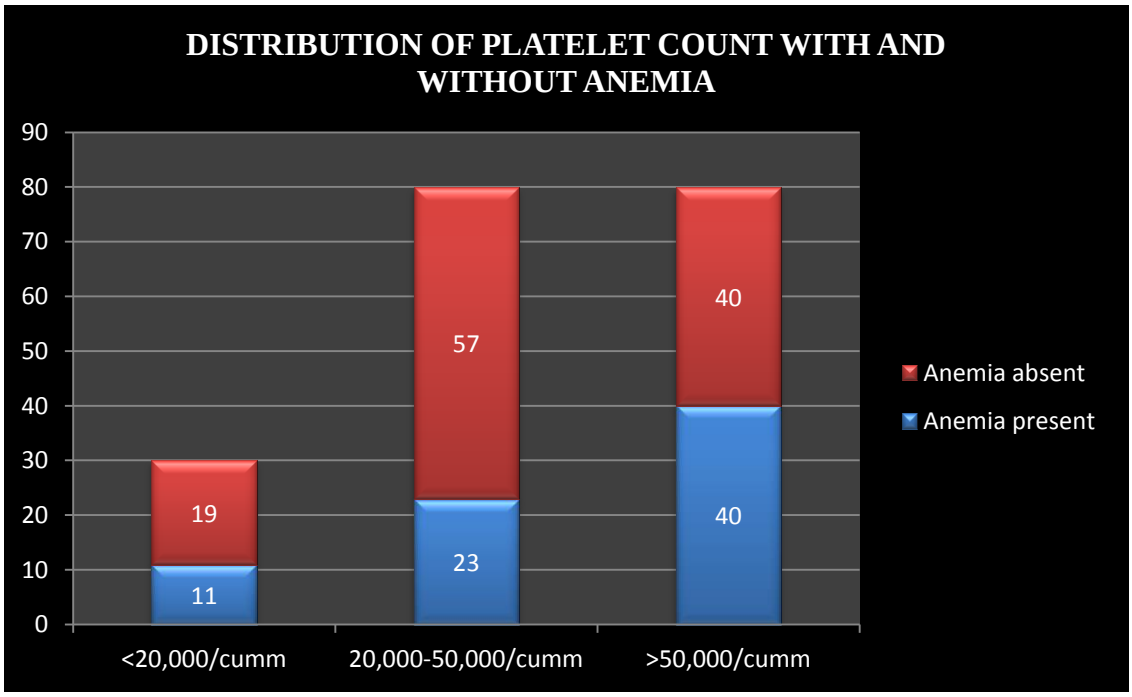


Figure: 16 Distribution of platelet count with anemia and without anemia.

Table 12: Comparison of bleeding manifestation with platelet indices

	Bleeding				P value
	Present		Absent		
	Mean	SD	Mean	SD	
MPV (fL)	5.7	1.3	7.5	1.6	<0.001*
PDW (fL)	15.1	7.9	19.0	6.2	<0.001*
PLCR (%)	24.7	5.9	27.1	9.2	0.035*
Platelet CRIB (%)	24.9	5.9	25.7	8.5	0.444

As shown in **table: 12,** mean MPV between cases with bleeding and without bleeding was 5.7± 1.3fL and 7.5± 1.6fL respectively and showed statistical significance with the p value of <0.001

Mean PDW between cases with bleeding and without bleeding was 15.1± 7.9fL and 19.0± 6.2fL respectively and showed statistical significance with the p value of <0.001

Similarly, it was observed that mean PLCR between cases with bleeding and without bleeding was 24.7± 5.9% and 27.1± 9.2% respectively and showed statistical significance with the p value of 0.035.

No significant difference was observed in mean plateletcrit between subjects with and without bleeding.

Table 13: Correlation between MPV, PDW, Plateletcrit and PLCR

		MPV	PDW	PLATELET CRIT	PLCR
Platelet Count	Pearson Correlation	0.275**	0.273**	0.300**	0.217**
	P value	<0.001*	<0.001*	<0.001*	0.003*
	N	190	190	190	190

**. Correlation is significant at the 0.01 level (2-tailed).

As shown in **table: 13,** significant positive correlation was observed between Platelet count and MPV. i.e. with increase in platelet count there was increasing in MPV and Vice versa.

Significant positive correlation was observed between Platelet count and PDW. i.e. with increase in platelet count there was increasing in PDW and Vice versa.

Significant positive correlation was observed between Platelet count and Platelet Crit. i.e. with increase in platelet count there was increasing in Plateletcrit and Vice versa.

Significant positive correlation was observed between Platelet count and PLCR. i.e. with increase in Platelet count there was increase in PLCR.

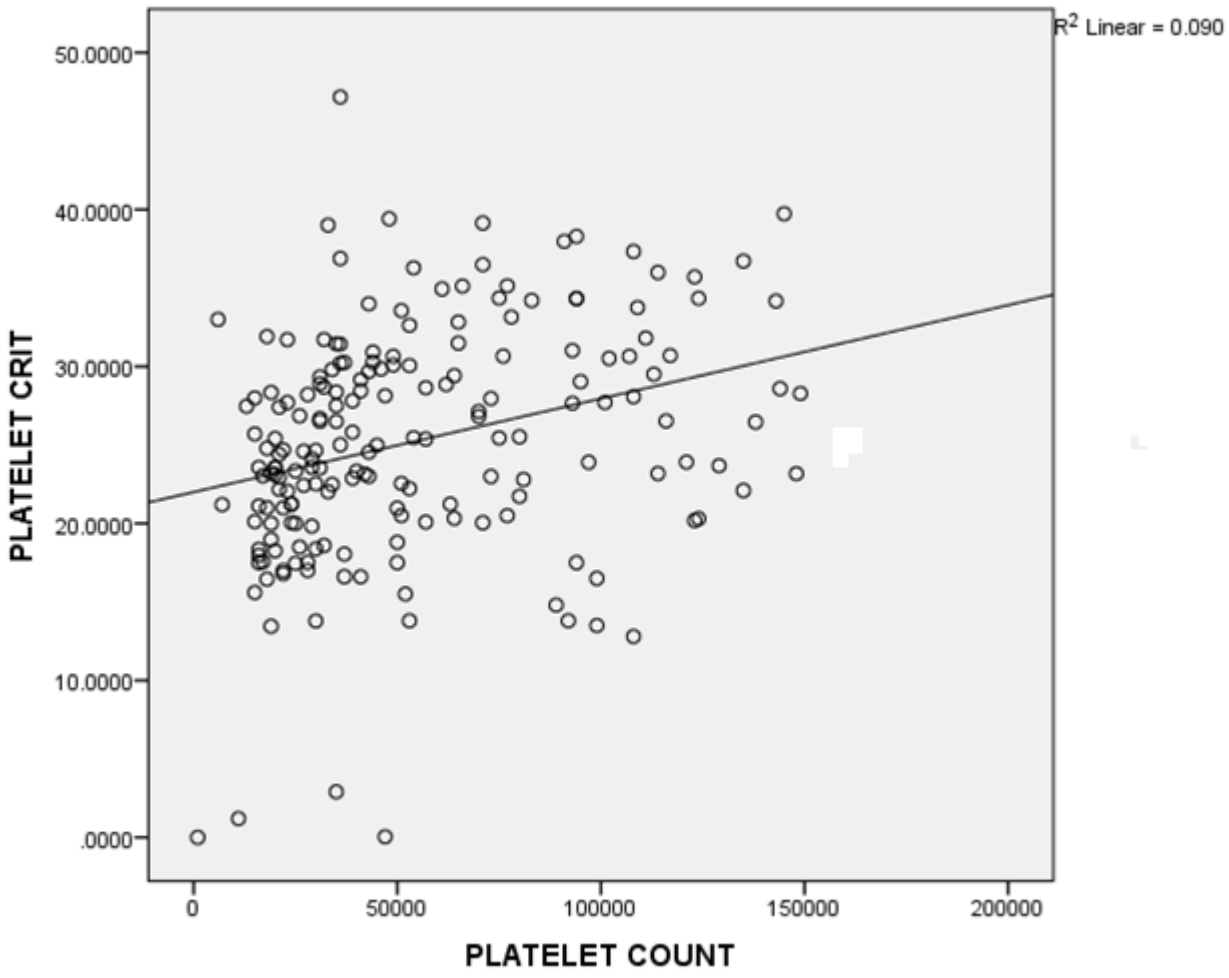


Figure 17: Scatter Plot showing Positive Correlation between Platelet Count and Platelet CRIT

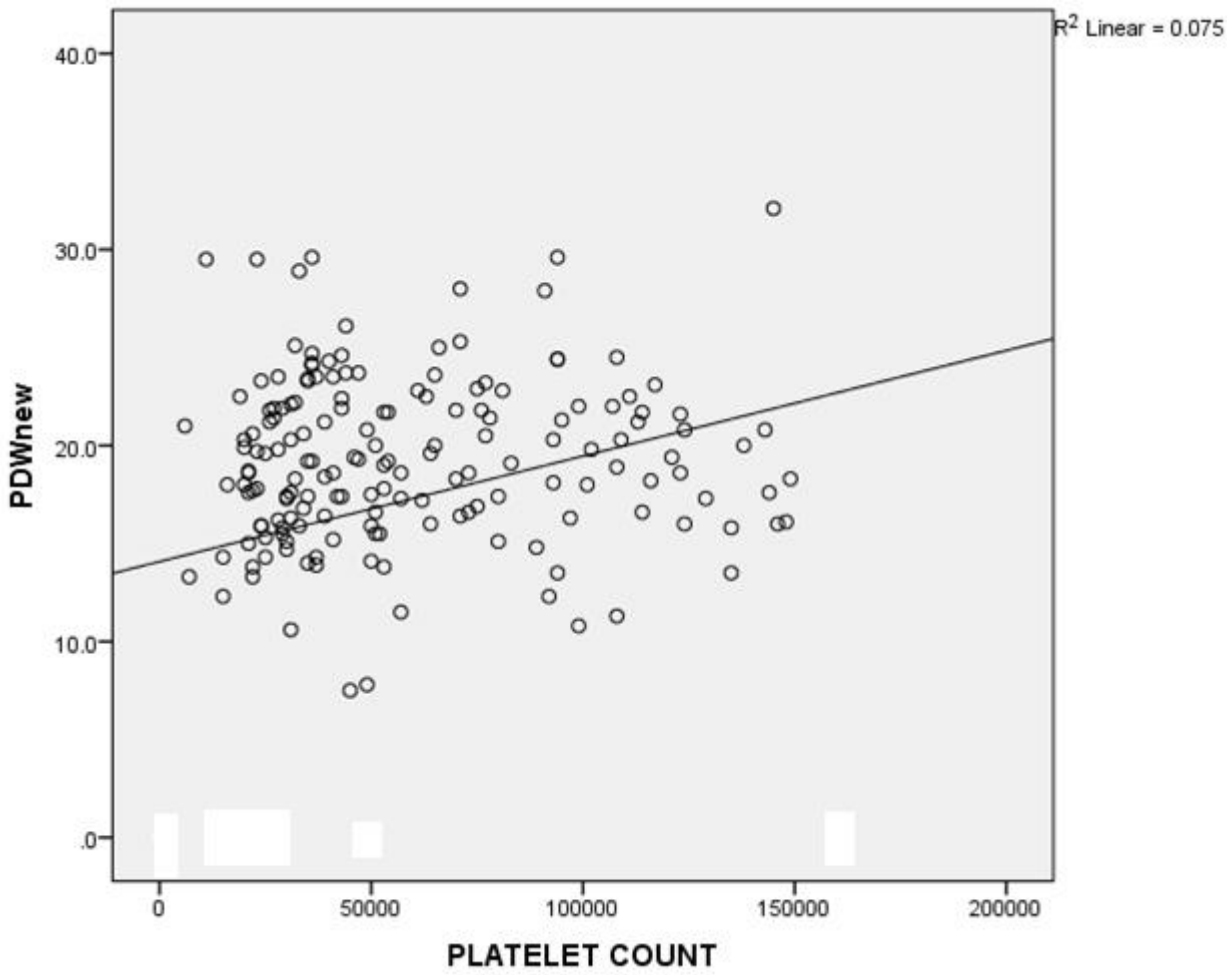


Figure 18: Scatter Plot showing Positive Correlation between Platelet Count and PDW

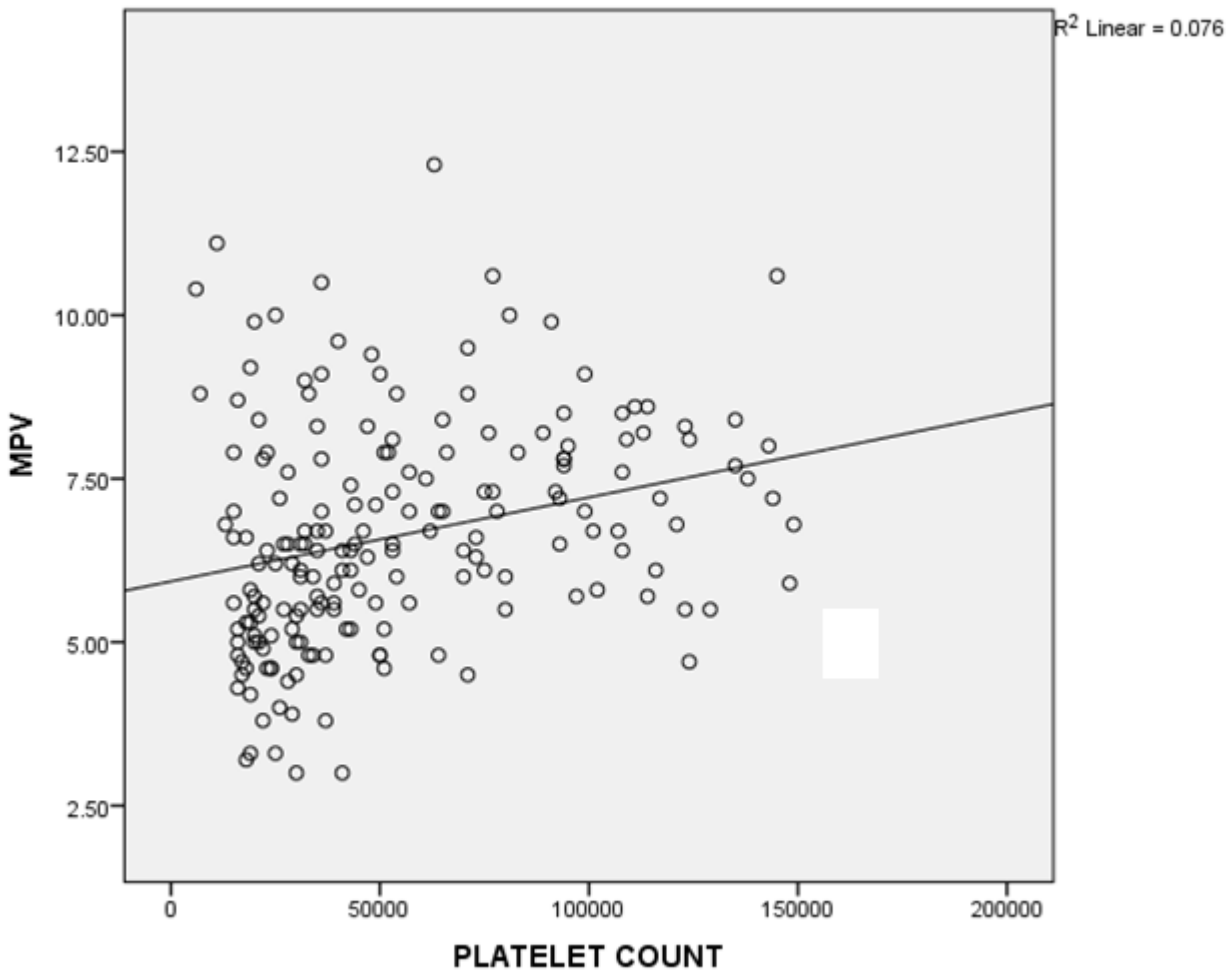


Figure 19: Scatter plot showing Positive correlation between MPV and platelet count

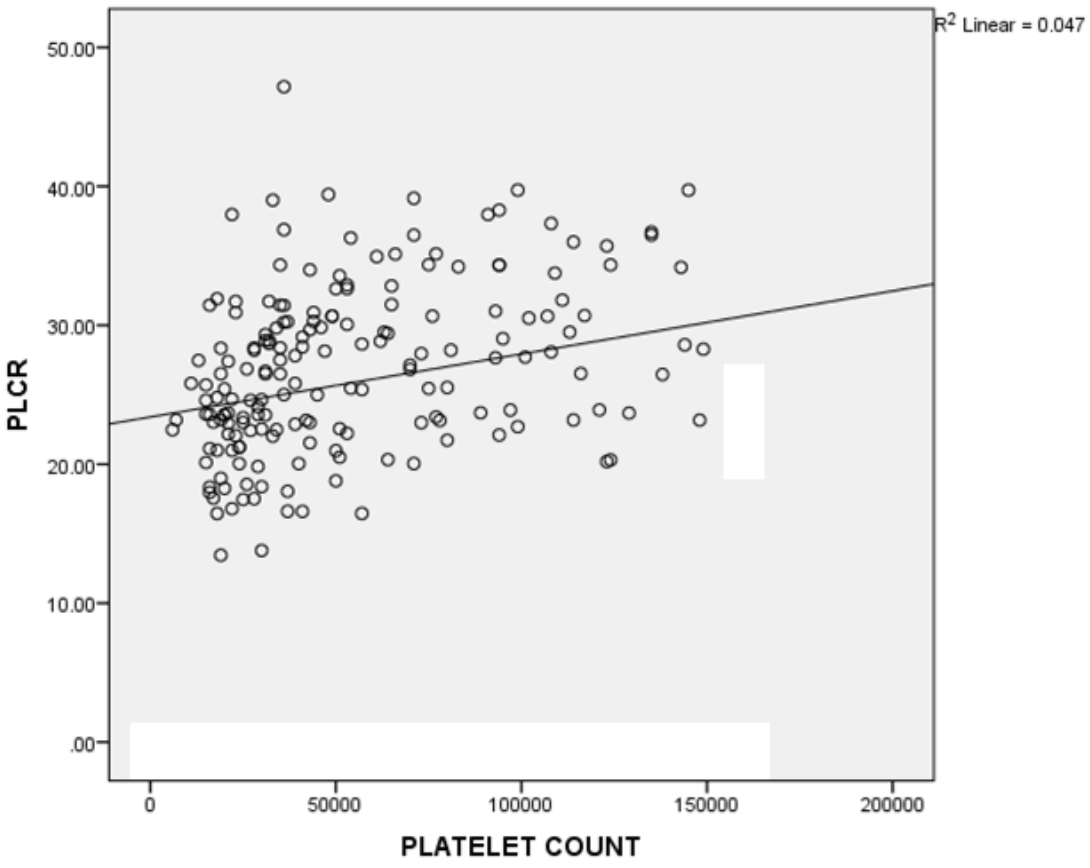


Figure 20: Scatter plot showing Positive correlation between MPV and PLCR

Table 14: Correlation between Platelet count, MPV, PDW and Platelet CRIT among subjects with bleeding and no bleeding.

		MPV	PDW	Plateletcrit	PLCR
Bleeding absent	Pearson Correlation	0.073	0.044	0.314**	0.155
	P value	0.483	0.674	0.002*	0.134
	N	95	95	95	95
Bleeding Present	Pearson Correlation	0.307**	0.407**	0.265**	0.244*
	P value	0.003*	0.001*	0.009*	0.017*
	N	95	95	95	95

As shown in **table: 14,** among subjects with bleeding, significant positive correlation was observed between Platelet count and MPV, PDW, plateletcrit and PLCR. i.e. with decrease in platelet count there was decrease in MPV, plateletcrit, PDW and PLCR and Vice versa.

Whereas, among subjects with no bleeding significant positive correlation was observed between platelet count and plateletcrit only.

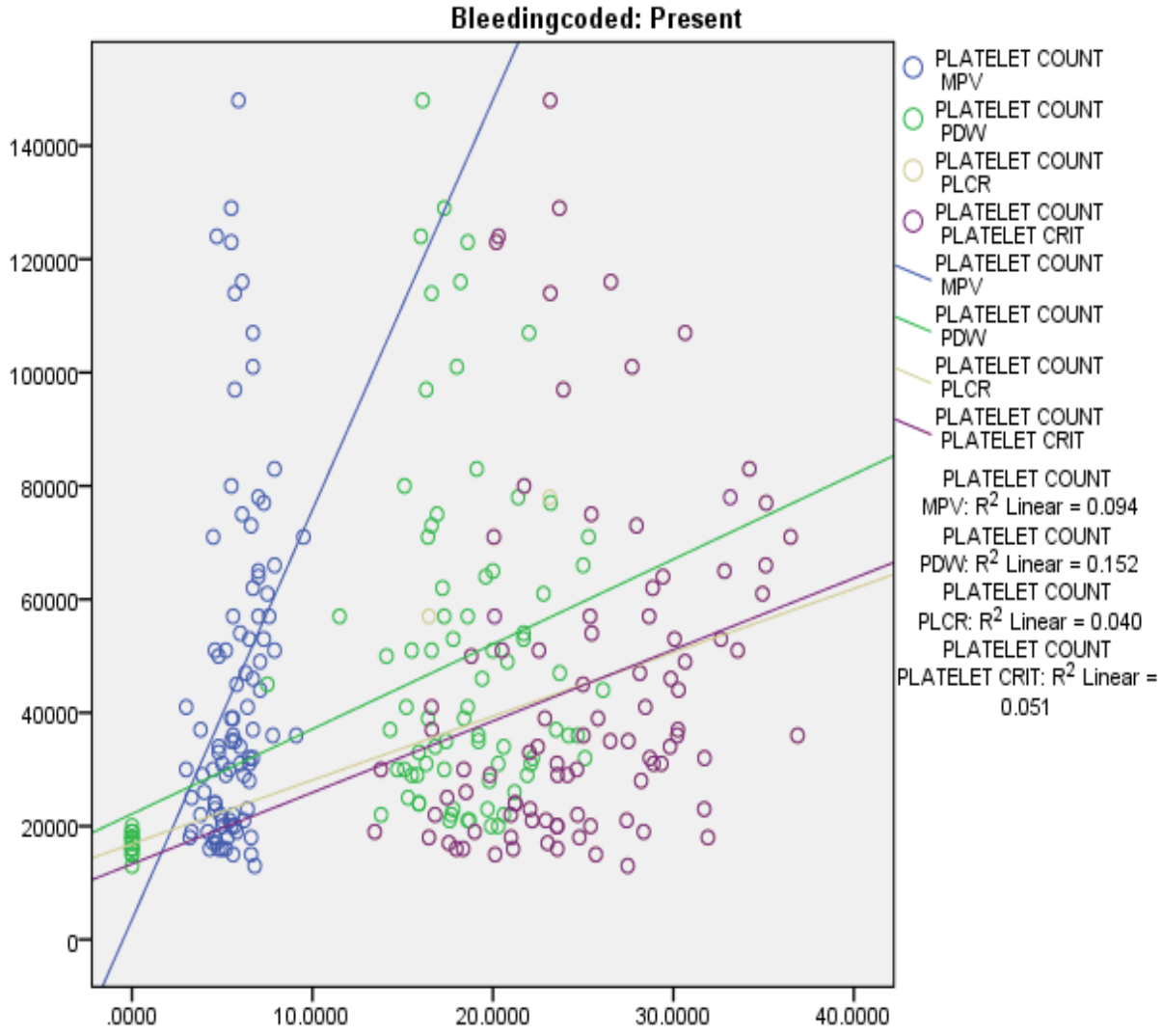


Figure 21: Overlay Scatter plot showing correlation between Platelet count, MPV, PDW and Plateletcrit among subjects with bleeding

Table 15: Distribution of Bleeding and Anemia with respect to platelet count

				ANEMIA				P value
				Absent		Present		
				Count	%	Count	%	
Platelet	< 20,000/cumm	Bleeding	Present	14	73.7%	6	54.5%	0.284
			Absent	5	26.3%	5	45.5%	
	20,000 to 50000/cumm	Bleeding	Present	36	63.2%	8	34.8%	0.021*
			Absent	21	36.8%	15	65.2%	
	> 50,000/cumm	Bleeding	Present	17	42.5%	14	35.0%	0.491
			Absent	23	57.5%	26	65.0%	

As shown in **table: 15** and **figure:22**, anemia was not significantly associated with bleeding manifestations, when platelet counts were <20,000/cumm and > 50,000/cumm. Whereas, significant association was observed in bleeding manifestations and anemia at 20,000 – 50000/cumm platelet count. i. e. among 63.2% of subjects with bleeding had no anemia when platelet count was 20,000 - 50,000/cumm.

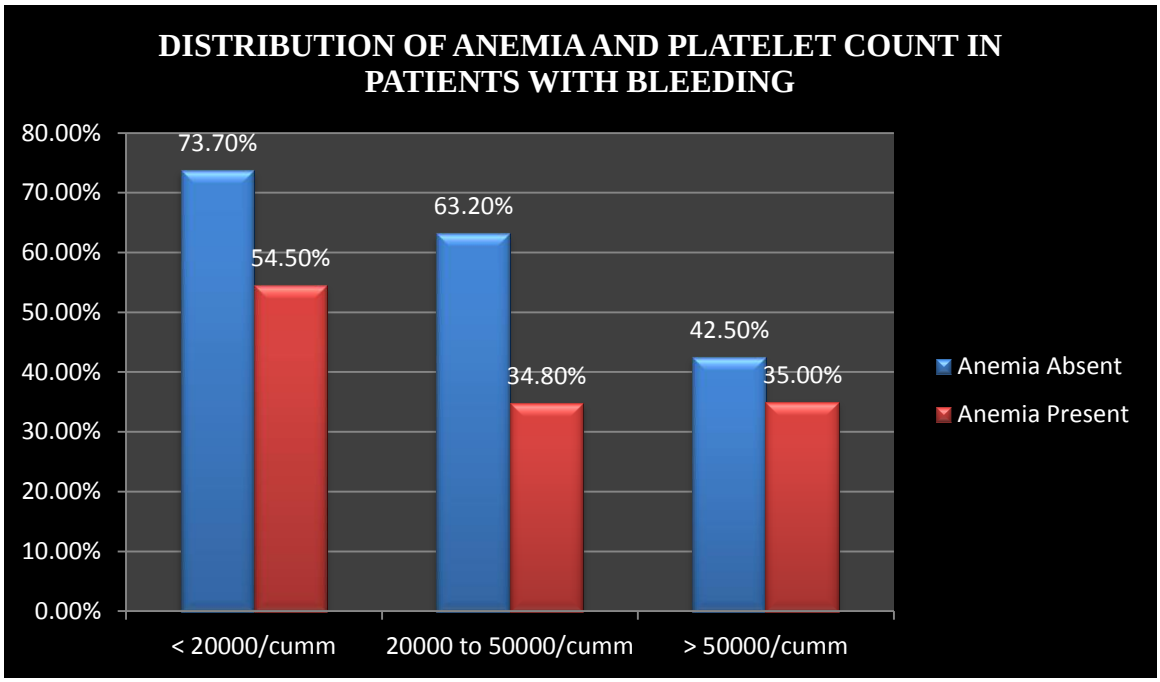


Figure 22: Distribution of Bleeding and Anemia with respect to platelet count (20,000 to 50,000)

Table 16: Mean MPV, PDW and Plateletcrit comparison among subjects with bleeding and without bleeding with respect to platelet count

				MPV			PDW			PLCR			PLATELETCRIT		
				Mean	SD	p	Mean	SD	p	Mean	SD	P	Mean	SD	p
Platelet	< 20000/cumm	Bleeding	Present	5.1	1.0	<0.001*	12.1	1.34	<0.001*	21.1	6.7	0.780	21.1	6.7	0.723
			Absent	8.3	2.0		14.9	9.3		21.9	8.4		20.1	8.4	
	20000 to 50000/cumm	Bleeding	Present	5.5	1.3	<0.001*	18.5	3.6	0.433	24.7	5.1	0.102	24.7	5.1	0.985
			Absent	7.0	1.6		19.4	6.0		27.1	8.0		24.6	8.8	
	> 50000/cumm	Bleeding	Present	6.5	1.1	<0.001*	18.7	3.1	0.374	27.1	5.5	0.592	27.5	5.3	0.963
			Absent	7.8	1.4		19.6	5.3		28.1	9.9		27.6	7.8	

As shown in **table: 16**, in all the three groups of platelet count there was significant difference in mean MPV between subjects with and without bleeding. I.e. Lower MPV was observed among those with bleeding at all the levels of platelet count.

In platelet count of <20000/cumm, significant difference in mean PDW was observed in subjects with and without bleeding. In other platelet groups there was no significant difference in mean PDW.

There was no significant difference in plateletcrit and PLCR between subjects with and without bleeding at all the levels of platelet count.

Table 17: Correlation between PLCR and Platelet indices

		PLCR	MPV	PDW	PLATELET CRIT
PLCR	Pearson Correlation	1	0.468**	0.425**	0.737**
	P value		<0.001*	<0.001*	<0.001*
	N	190	190	190	190

As shown in **table: 17**, significant positive correlation was observed between PLCR with MPV, PDW and Platelet crit. i.e. with decrease in PLCR there was decrease in MPV, PDW and Plateletcrit and vice versa.

Table 18: Correlation between PLCR and Platelet indices between subjects with and without bleeding

			PLCR	MPV	PDW	PLATELET CRIT
PLCR	Bleeding Present	Pearson Correlation	1	0.871**	0.518**	0.983**
		P value		0.001*	0.001*	0.001*
		N	95	95	95	95
	Bleeding Absent	Pearson Correlation	1	0.262*	0.354**	0.625**
		P value		0.011*	<0.001*	<0.001*
		N	95	95	95	95

As shown in **table: 18**, significant positive correlation was observed between PLCR with MPV, PDW and Platelet crit. I.e. with decrease in PLCR there was decrease in MPV, PDW and Plateletcrit and vice versa in both groups with and without bleeding.

DISCUSSION:

Platelets are disc shaped non nucleated organelle which plays an important role in hemostasis and thus thrombus formation.

Bleeding tendency increases as the platelet count decreases and thus bleeding is inversely related to platelet count. Thrombocytopenia can be due to decreased platelet production or increased platelet destruction.

Apart from platelet count, platelet indices that includes- MPV, PDW, Plateletcrit and PLCR have utility in predicting bleeding risk. Lower the platelet indices; more is the chance of bleeding.

Table: 19 Age and sex distribution:

	Navya B N et al	Raikar S et al	Cortelazzo et al	Present study
Age group	21-40 years	12-30 years	<40 years	21-30 years
Sex distribution	F >M	M>F	F>M	F>M

In the present study, the most common age group was 21 - 30 years in females and 31-40 years among males, while in a study done by Raikar S et al [49], majority of cases were in the age group was 12-30 years ,according to Navya B N et al [48], majority of cases in the age group of 21-40 years and according to Cortelazzo et al, most common age group was <40 years.

In the present study, majority were females except for the age group of <20 years. However, there was no significant association between gender, platelet count and bleeding manifestation. While in study done by Raikar S et al[49] and Usha rani et al [50], males were more commonly

affected than females, according to Navya B N et al[48] and Cortelazzo et al, females were more commonly affected.

Table: 20 Female to male ratio

	Usha rani et al	Cortelazzo B S et al	Present study
F:M Ratio	1:2	1:2.9	1.375:1

In the present study, Female: male ratio was 1.375:1 while in a study done by Usha rani [50] et al and Cortelazzo B S et al [38], female: male ratio was 1:2 and 1:2.9 respectively.

Etiology of thrombocytopenia-

The most predominant cause of thrombocytopenia in the present study is acute febrile illness followed by obstetric cause and leukemia which is similar to other study done by Patil P et al, where febrile illness was the most common.[60]This is mainly due to the fact that the most common cause of thrombocytopenia being fever with infectious cause, mainly viral induced thrombocytopenia.

Table 21: Bleeding distribution

	Kumar et al	P S Nair et al	Gandhi et al	Puttu Suresh et al	Modi et al	Usha rani et al	Bhashir et al	Present study
Gum bleed	5.2%	13.3%	16.28%	8%	6.21%	12%	2.7%	29.4%
Petechiae	67.2%	22.2%	76.74%	16%	67.23%	2%	-	27.3%
Melena	4.7%	-	-	12%	9.03%	32%	-	14.7%
Hematuria	-	-	1%	8%	1.12%	16%	5.7%	16.8%
Hematemesis	3%	-	-	12%	1.12%	-	0.3%	4.2%
Epistaxis	2.6%	15.56%	-	2%	7.90%	-	1.5%	17.9%

In the present study, out of 95 patients with bleeding, the most common bleeding manifestations were gum bleed followed by petechiae. However, most studies had presence of petechiae as the most common bleeding manifestation in study done by Kumar et al [51], P S Nair et al [52], Gandhi et al[53], Puttu Suresh et al[54] and Modi et al[55].

In the present study, the bleeding manifestations included bleeding gums- 28 cases, blood in stools 14 cases, hematuria 16 cases, bleed from I/V cannula 3 cases, conjuctival bleed 9 cases, 4 hematemesis, 6 petechiae skin, 20 petechiae of oral cavity and epistaxis 17 cases.

In a study done by Kumar A et al[51], most common bleeding manifestations were petechiae (67.2%), gum bleed (5.2%), melena (4.7%), haemetemesis (3%) and epistaxis (2.6%).[51]

In a study done by PS Nair et al, petechiae / purpura was the commonest bleeding manifestations (22.22%, n=45) and GI bleed (22.22%) followed by epistaxis (15.56%, n=45), bleeding gums (13.33%, n=45), and other bleeding accounting for 26.67 %(n=45).[52]

In a study done by Gandhi AA et al the most common bleeding manifestation was petechiae (76.74%) followed by bleeding gums (16.28%).[53]

In a study done by Putta Suresh the commonest bleeding manifestation was cutaneous in the form of petechiae and purpura, which was seen in 8 cases (16%), followed by hematemesis (12%), malena (12%), bleeding gums (8%), hematuria (8%) and epistaxis (2%).[54]

According to Modi et al, the most common bleeding manifestation was petechiae 67.23% followed by melena (9.03%), epistaxis (7.9) and gum bleed (6.21%).[55]

According to Usha Rani et al, melena (32%) was the commonest symptom, followed by hematuria (16%) and gum bleed (12%).[50]

According to Basir et al, among patients with bleeding, most common was hematuria (5.7%) followed by gum bleed (2.7%).[56]

TABLE- 22 Platelet count distribution with bleeding and without bleeding.

PLATELET COUNT	BLEEDING PRESENT			BLEEDING ABSENT		
	Usha rani et al	Basir et al	Present study	Usha rani et al	Basir et al	Present study
<20,000/cumm	23 (78.7%)	68.5%	20 (21.1%)	4 (23.5%)	18%	10 (10.5%)
20,000-50,000/cumm	8 (24.2%)		44 (46.3%)	7 (41.3%)		36 (38%)
>50,000/cumm	2 (6.1%)	31.5%	31 (32.6%)	6 (35.2%)	82%	49 (51.5%)

In the present study, out of 95 cases of thrombocytopenia with bleeding, 20 cases (21.1%) fall under the category of platelet count<20, 00/cumm, 44 cases (46.3%) with platelet count 20,000-50,000/cumm and 31 cases (32.6%) with platelet count >50,000/cumm i.e. maximum cases with bleeding had platelet count of range between 20,000-50, 00/cumm like another study by Basir et al. According to Usha Rani et al[50], bleeding was maximum in the cases with platelet count <20,000/cumm (78.7%).

Whereas, in thrombocytopenic cases without bleeding, in the present study, out of 95 cases, 10 cases (10.5%) fall under the category of <20,000/cu mm, 36 cases (38%) with platelet count between 20,000- 50,000/cu mm and 49 cases(51.5%) of platelet count >50,00/cu mm i.e. maximum number of cases without bleeding fall under the category of platelet count >50,000/cumm unlike another study done by Usha Rani et al [50] where, without bleeding cases were more in 20,000-50,000/cumm (41.3%).

In the present study among subjects who presented with bleeding mean platelet count was 44,752.6 ± 30,039.6 and among without bleeding subjects was 66,268.8 ± 41,144.8.

Table: 23 Distribution of platelet count

Platelet count	PS Nair et al	Puttu Suresh et al	Nakhale B D et al	Bhalara A et al	Usha Rani et al	Present study
<20,000/cumm	17.4%	56.25%	10.65%	16.7%	54%	13.7%(n=26)
20,000-50,000/cumm	25.8%		27.86%	23.5%	30%	45.2%(n=86)
>50,000/cumm	56.8%	43.75%	61.46%	59.8%	16%	41.1%(n=78)

In the present study, 13.7% cases had platelet count <20,000/cu mm, 45.2% had platelet count in the range between 20,000- 50,000/cu mm and 41.1% cases had platelet count >50,000/cu mm.

In a study, conducted by Nair PS et al, 56.8% had platelet count > 50,000/cu mm followed by 28 patients (25.7%) between 20,000 to 50,000,and 9.2% between10,000 to 20,000 , and 8.2% had <10,000.[52] In Gandhi AA e al study platelet count in the range of 50,000-1,50,000cu mm was seen in 57.14%, platelet count in the range of 20,000- 50000cu mm was seen in 29.47% and platelet count <20000cu mm was seen in 13.39%.[53] In the study by Puttu Suresh et al, 15% cases were seen with platelet count<20,000/cu mm, 13.3% cases with platelet count in the range between 20,000-50,000/cu mm and rest 64% cases having platelet count >50,000/cu mm.[54] In Bhalara et al study 1,00,000- 1,50,000 present in 31.4%, 50,000 - 1,00,000 present in 28.4%, 20,000-50,000 present in 23.5%, 10,000-20,000 present in 9.7% and <10,000 present in 6%.[58]

In study done by Usha Rani et al, 54% cases had platelet count <.20,000/cumm, 30% cases had platelet count between 20,000-50,000/cumm and rest 16% had platelet count >50,000/cumm.[50]

Table: 24 Comparison of platelet count and MPV in patients with bleeding and without bleeding.

	Bleeding present		Bleeding absent	
	Kennet et al	Present study	Kennet et al	Present study
Platelet count ($X10^3$/cumm)	27..4± 22.0	66.2± 41.14	47.1± 21.0	44.52± 30.2
MPV (fL)	6.83± 1.89	5.7± 1.3	8.98± 1.13	7.5± 1.6

In the present study, Mean Platelt count in thrombocytopenic patients with bleeding was 66,000/cumm and without bleeding was 44,000/cumm. While, in a study done by Kennet et al, mean platelet count with bleeding was 27,000/cumm and without bleeding was 47,000.cumm.

In the present study, Mean MPV count with bleeding was 5.7 fL and without bleeding was 7.5 fL while, in Kennet et study [59], mean MPV with bleeding and without bleeding was 6.83 and 8.98 fL respectively.

Table: 25 Comparision of Mean platelet count and PDW:

	Bashir et al	Present study
Mean Platelet count	95,691/cumm	54,482/cumm
Mean PDW	15.56fL	18.36fL

According to study done by Bashir et al[56], mean platelet count was 95,691/cumm and mean PDW was 15.56fl while, in the present study, mean platelet count was 54,482/cumm and mean PDW was 18.36fL.

Table : 26 Distribution of bleeders and non bleeders

	Patil et al	Srinivas et al	Present study
Bleeding present	23%	49%	50%
Bleeding absent	77%	51%	50%

In the present study, 50% cases had bleeding and 50% without bleeding. While in a study done by Patil et al [60], 23% had bleeding and 77% without bleeding and according to Srinivas et al [61] study, 49% cases had bleeding and 51% presented without bleeding. However, the present study was limited by the case selection as cases of thrombocytopenia with and without bleeding were taken in 1:1 ratio.

Table:27 Corelation of platelet indices and etiology

	Negash et al		Elsewefy et al		Present study	
Etiology	Hypoproductive	ITP	Hypoproductive	ITP	Bleeding present	Bleeding absent
Platelet count(/cumm)	70,000	72,400	45,300	35,800	44,752	66,268
MPV (fL)	9.7	12.4	9.08	9.97	5.7	7.5
PDW (fL)	13.2	15.5	16.90	17.11	15.1	19.0
PLCR (%)	25	36.8	27.57	41.68	24.7	25.7

In the present study, Mean platelet count with and without bleeding was 44,752 and 66,268 respectively. Mean MPV, PDW and PLCR with bleeding was 5.7fL, 15.1fL, 24.7 % respectively and Mean MPV, PDW and PLCR without bleeding was 7.5fL, 19.0fL and 25.7% respectively.

While in a study done by Negash et al[47], mean Platelet count, mean MPV, mean PDW and mean PLCR in hypoproductive state was 70,000/cumm, 9.7 fL, 13.2fL and 25% respectively and in ITP cases mean Platelet count, mean MPV, mean PDW and mean PLCR was 72,400/cumm, 12.4fL, 15.5fL and 36.8% respectively.

In another study done by Elsewefy et al [62], mean Platelet count, mean MPV, mean PDW and mean PLCR in hypoproductive state was 45,300/cumm, 9.08 fL, 16.90 fL and 27.57% respectively and in ITP cases mean Platelet count, mean MPV, mean PDW and mean PLCR was 35,800/cumm, 9.97fL, 17.11fL and 41.68% respectively.

In the present study, lower platelet count and indices were seen in patients with bleeding and compared to the study done by Elsewefy et al [62], there platelet indices were low in hypoproductive thrombocytopenia.

This suggests that increased risk of bleeding in patients with low platelet indices would be due predominantly to hypoproductive phenomena than just destructive phenomena and also suggesting reduced platelet indices to be associated with increased activation of platelets and prothrombotic effect. Therefore, low platelet indices leads to increased tendency of bleeding.

The above finding support the hypothesis that larger platelets are enzymatically more active and hence to with similar platelet count but low platelet indices are more prone to bleed than ones with raised platelet indices.

However, direct comparision between other studies could not be done with regard to platelet indices such as PDW, P-LCR due to the paucity of studies on impact of platelet indices with risk of bleeding in patients with thrombocytopenia.

CONCLUSION:

Alterations in platelet indices helps predict the bleeding tendency as in patients of thrombocytopenia with bleeding, platelet indices (MPV, PDW, Plateletcrit and PLCR) were low whereas in patients of thrombocytopenia without bleeding, platelet indices (MPV, PDW, Plateletcrit and PLCR) were high. The risk of bleeding was common in patients with infectious etiology of thrombocytopenia compared to other causes.

With decrease in platelet count, PDW, Plateletcrit and PLCR were low in patients of thrombocytopenia with bleeding. Larger platelets with high PLCR had low risk of bleeding.

In our study, statistical significance was found between MPV, PDW, PLCR and bleeding.

Patients with raised platelet indices indicates functionally active platelets and the cause of thrombocytopenia been predominantly the platelet destruction while, among patients with low platelet indices, the frequent cause of thrombocytopenia is decreased platelet production.

Thus, in the present study, the common cause of thrombocytopenia with bleeding was hypoproductive phenomena rather than detructive phenomena as in thrombocytopenic patients with bleeding, all platelet indices were low.

However, further studies need to be done with individual etiologies and multiple values of platelet indices during the course of patients in hospital to see direct role of platelet indices in bleeding.

SUMMARY:

In our study of 190 patients of thrombocytopenia, 95 with bleeding and 95 without bleeding, we found that:

1) In the present study, 21-30 years was the most common age group and among that females were most frequently affected than males.Females to male ratio was 1.375:1.
2) The most common etiology of thrombocytopenia in the present study was infectious etiology and the most common bleeding manifestation was gum bleed followed by petechiae.
3) Majority of thrombocytopenic patients with bleeding had platelet count in the between 20,000-50,000/cumm.
4) Mean platelet count in patients who presented with bleeding was 44,752/cumm and among without bleeding subjects was 66,268/cumm.
5) Lower the platelet count all platelet indices including MPV, PDW, Plateletcrit was low in patients of thrombocytopenia with bleeding.
6) With decrease in platelet count, P-LCR also decreased in patients of thrombocytopenia with bleeding.
7) Mean MPV among those with bleeding was 5.7fL and without bleeding was 7.5fL.
8) Mean PDW among those with bleeding was 15.1 and without bleeding was 19.0
9) Mean PLCR among those with bleeding was 24.7 and without bleeding was 27.1.
10) Mean Plateletcrit among patients with bleeding was 24.9% and without bleeding was 25.7%.

11) All mean platelet indices (MPV, PDW, Plateletcrit and PLCR) were lower in thrombocytopenic patients with bleeding than patients without bleeding.

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ANNEXURE: I

PATIENT PROFORMA

Serial number: Date:

Hospital number: Address:

Name:

Age:

Gender:

Bleeding:Present/Absent:

Hb:

Clinical diagnosis:

Platelet count:

MPV:

PDW:

PLCR:

Platelet crit:

PT:

apTT:

ANNEXURE: II

INFORMED CONSENT FORM

I,the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information as outlined in this consent form.

I understand the purpose of this study, the risks and benefits of the two techniques and the confidential nature of the information that will be collected and disclosed during the study. The information collected will be used only for research.

I have had the opportunity to ask questions regarding the various aspects of this study and my questions have been answered to my satisfaction.

I understand that I remain free to withdraw from this study at any time and this will not change my future care.

Participation in this study does not involve any extra cost to me.

Subject's name and signature /thumb impression Date:

Name and signature of witness Date:

Name and signature of person obtaining consent Date:

ಮಾಹಿತಿಯುಕ್ತಸಮ್ಮತಿಯನಮೂನೆ

ನಾನು ರುಜು ಮಾಡಿರುವ, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಈಸಮ್ಮತಿಯ ರೂಪಅಂಶಗಳಂತೆ ನನ್ನ ವೈಯಕ್ತಿಕ

ಮಾಹಿತಿಯ ಸಂಗ್ರಹಣೆ ಮತ್ತು ಪ್ರಕಟಣೆ ಅಧಿಕೃತಗೊಳಿಸಲು ಒಪ್ಪುತ್ತೇನೆ.

ನಾನು ಈ ಅಧ್ಯಯನದ ಕಾರಣ ,ಅರ್ಥ, ಅಪಾಯಗಳು ಮತ್ತು ಲಾಭಗಳ ಎರಡು ತಂತ್ರಗಳನ್ನು ಮತ್ತು ಸಂಗ್ರಹಿಸಿ

ಅಧ್ಯಯನಮಾಡುವಸಂದರ್ಭದಲ್ಲಿಬಹಿರಂಗಪಡಿಸಲಾಗುತ್ತದೆಮಾಹಿತಿಯನ್ನುಗೌಪ್ಯಪ್ರಕೃತಿ.

ಸಂಗ್ರಹಿಸಿದಮಾಹಿತಿಯನ್ನುಮಾತ್ರಸಂಶೋಧನೆಗೆಬಳಸಲಾಗುತ್ತದೆ.

ನಾನುಈಅಧ್ಯಯನದಲ್ಲಿವಿವಿಧಅಂಶಗಳನ್ನುಕುರಿತುಪ್ರಶ್ನೆಗಳನ್ನುಕೇಳಲುಅವಕಾಶಹೊಂದಿದ್ದರುಮತ್ತುನನ್ನಪ್ರಶ್ನೆಗಳಿಗೆನ

ನ್ನತೃಪ್ತಿಉತ್ತರಗಳನ್ನುನೀಡಲಾಗಿದೆ.

ನಾನುಯಾವುದೇಸಮಯದಲ್ಲಿಈಅಧ್ಯಯನದಿಂದಹಿಂದಕ್ಕೆಪಡೆಯಬಹುದುಉಳಿದುಈನನ್ನಭವಿಷ್ಯದಕಾಳಜಿಬದಲಾಗು

ವುದಿಲ್ಲಎಂದುಅರ್ಥ.

ಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸುವಿಕೆನನಗೆಯಾವುದೇಹೆಚ್ಚುವರಿವೆಚ್ಚವಿಲ್ಲದೆಒಳಗೊಳ್ಳುವುದಿಲ್ಲ.

ವಿಷಯದಹೆಸರು :

ಸಹಿ / ಹೆಬ್ಬೆಟ್ಟಿನಗುರುತು : ದಿನಾಂಕ:

ANNEXURE III

PROCEDURE FOR PT

Blood is collected in a vacutainer with 3.2% sodium cutrate as anticoagulant in ratio of 1:9

Blood is centrifuged for 10 min at 2500g.

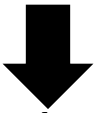
Plasma is collected in plastic tubes.

The cuvette strips are placed in a incubation area for prewarming at 37' C for 3 mins.

After incubation of the cuvette, 50 micro litre of plasma is dispensed into the cuvette and the timer is started for an incubation period of 60 secs.

When the instruments starts to beep, the cuvettes are transferred into the test column area.

100 microliter reagent of PT prewarmed at 37%c is added pressing the pipette key.



PT results are btained and are expressed in seconds.

PROCEDURE FOR aPTT

Blood is collected in a vacutainer with 3.2% sodium cutrate as anticoagulant in ratio of 1:9

Blood is centrifuged for 10 min at 2500rpm

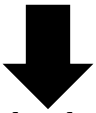
Plasma is collected in plastic tubes.

The cuvette strips are placed in a incubation area for prewarming at 37' C for 3 mins.

After incubation of the cuvette, 50 micro litre of plasma and 50micro litre of aPTT reagent is dispensed into the cuvette and the timer is started for an incubation period of 180 secs.

When the instruments starts to beep, the cuvettes are transferred into the test column area.

50 microlitre of $Cacl_2$ prewarmed at 37 ^{o}C is dispensed after pressing the pipette key.



aPTT results are obtained and are expressed in seconds.

ANNEXURE IV

KEY TO MASTER CHART

AFI - ACUTE FEBRILE ILLNESS

MPV - MEAN PLATELET VOLUME

PDW - PLATELET DISTRIBUTION WIDTH

P-LCR - PLATELET TO LARGE CELL RATIO

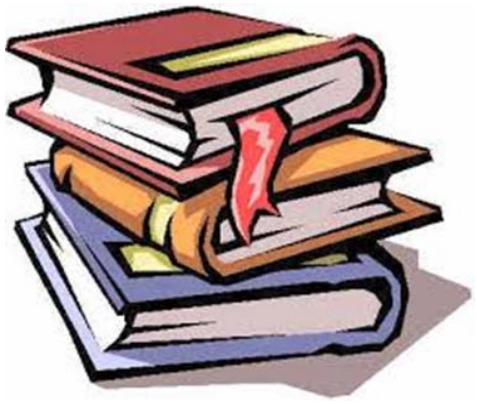
COLUMN C

0 - FEMALE

1 - MALE

Introduction

Objectives



Review of Literature

Materials & Methods

Observations & Results

Discussion

Conclusion

Summary

Bibliography

Annexures