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# Utility of Platelet Indices to Differentiate Reactive vs Clonal Thrombocytosis

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**Abstract:** ***Introduction: Thrombocytosis can be due to various causes. Modern hematology analyzers estimate various platelet indices including platelet count, MPV (mean platelet volume), PCT (plateletcrit) and PDW (platelet distribution width). Platelet indices have been used to differentiate various causes of thrombocytosis. This study was undertaken to assess the utility of platelet indices. Materials and Methods: Patients who presented to the Pathology department, for bone marrow studies, with persistent platelet count ≥ 4.5x10$^9$ /L were included in the study, along with 20 normal controls. Correlating the clinical features, peripheral blood and marrow studies, cases of thrombocytosis were classified as reactive (20) and clonal (40). These were compared with the normal (20) group. The various platelet indices, like platelet count, MPV, PCT and plateletcrit were estimated using automated hematology analysers. The data was analysed using unpaired t-test and chi square test. Results: There was no significant difference of platelet counts between the reactive and clonal groups. The values of PDW and plateletcrit were found to be significantly higher in the clonal group than the reactive group. Though the MPV values of all groups are within normal limits the mean values are significantly higher in the clonal group compared to reactive group. Conclusion: MPV, PDW and PCT values were found to be significantly higher in clonal group when compared to reactive groups. Platelet indices may help us to predict whether thrombocytosis is of reactive or clonal etiology, hence helping us to decide further ancillary tests.***

**Keywords:** Thrombocytosis, Platelet indices, Reactive, Clonal

## 1. Introduction

Thrombocytosis refers to abnormally higher number of platelets in the peripheral blood. The value greater than 4.5 lakhs/µl is considered as thrombocytosis and it is generally accepted.[1] The differential diagnosis of thrombocytosis is not always obvious, since multiple causes may be exist. This has both diagnostic and therapeutic implications**.** The routine clinical pathology laboratory classically provides only limited help in distinguishing between reactive thrombocytosis (RT) and clonal thrombocytosis

Clonal thrombocytosis is a disorder[1], caused by abnormal and uncontrolled expansion of hematopoietic cells. In contrast, Reactive or secondary thrombocytosis (RT) is caused by stimulated megakaryopoiesis because of various haematological or non-hematological disorders. The need for distinction between reactive and autonomous thrombocytosis resides in the increased incidence of thrombo hemorrhagic complications in the clonal group, and the fact that they can progress to acute leukemia.

A few studies done earlier have shown that some of the platelet indices can be used to differentiate various causes of thrombocytosis. [2,3,4]

Various platelet indices are Platelet count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Plateletcrit (PCT).

There are not many studies from India using platelet indices. [5,6,7,8,9] Hence we decided to explore the clinical utility of these platelet indices which are not regularly used, in the differential diagnosis of thrombocytosis, by using modern automated analysers.

### Aims and Objectives

- To determine if the various platelet indices can be used in the differential diagnosis of thrombocytosis.
- To compare results with the clinical features /peripheral blood and bone marrow studies.
- To correlate with cytogenetics/ molecular studies, wherever available.

## 2. Materials and Methods

The study included 60 cases of thrombocytosis of varying etiologies. Twenty samples from healthy people were included as controls. The study began in October 2011 and was completed in June 2013. All patients presenting to the pathology department, NIMS for bone marrow studies, with persistent platelet count ≥ 4.5 lakhs/cumm were included in the study. The clinical details including follow-up details were obtained from the patient's medical records. Apparently normal patients with normal platelet count were taken as controls. Patients with count ≥ 4.5 lakhs/cumm without bone marrow study were excluded. Ethics approval was taken.

### Procedures done in the Pathology Department

**Bone marrow aspiration-** The preferred site was the posterior superior iliac spine. EDTA(1%) was added to prepare films and to prevent clotting of bone marrow aspirate. During the procedure material is collected for cytogenetics or immunophenotypic analysis when required. For the trephine biopsy a larger needle (Jamshidi needle) was used. The biopsy was preserved in B5 fixative and prepared for furthur processing with 9.5% $HNO_3$ in 1% EDTA which used as a decalcifying agent and after adequate decalcification, routine tissue processing was done and the block prepared.

### Blood Investigations

Venous blood was collected in $K_3$EDTA and complete haemogram, was done for all cases including platelet indices like platelet count, Mean platelet volume, plateletcrit, platelet distribution width using Beckman Coulter LH 500 and DxH 800.

**Peripheral Smear & bone marrow study** - The peripheral smears done at the time of procedure, marrow aspirates & imprints were reviewed. Peripheral smears and imprints were stained with Giemsa stain, and all the biopsies were stained with H & E, Reticulin stain, & if necessary Masson Trichrome (for fibrosis). Cytogenetics reports were obtained for 45cases and JAK2 mutational analysis was available for 4 cases.

**Statistics:**
The values of platelet indices were collected and statistically analysed using unpaired t- test. A P- value of 0.05 or less was considered statistically significant.

## 3. Results

We studied 60 cases of thrombocytosis with an age ranging from 4 - 70 years, the mean age being 39.2 years. Males constituting 29, females constituting 31 cases with a M: F ratio is 1:1.06. Out of 60 cases of thrombocytosis, 40 were diagnosed to be of clonal etiology and 20 of reactive causes.

Twenty apparently healthy individuals with normal hemograms were included in study as controls. Their ages ranged from 20-75 years with mean age of 46.6 years. These included 8 males and 12 females.

**Demography of Reactive cases**
Reactive cases include 20 cases of which 11 are males and 9 were females. Based on bone marrow study and other clinical, biochemical data, the etiologies of reactive group were classified as : Iron deficiency anemia- 6 cases. Inflammatory-5 cases- all had some form of arthritis. Malignancy- 4 cases- These included 2 cases of nodal NHL without marrow involvement, 1 case of Hodgkin lymphoma with marrow involvement & 1 with marrow involvement by neuroblastoma. Infections- 2 cases of infectious etiology, one showing granulomas (possibly of Koch etiology) in the marrow. There was one case each of reactive thrombocytosis associated with cardiomyopathy, megaloblastic anemia and a treated AML patient, status post-chemotherapy.

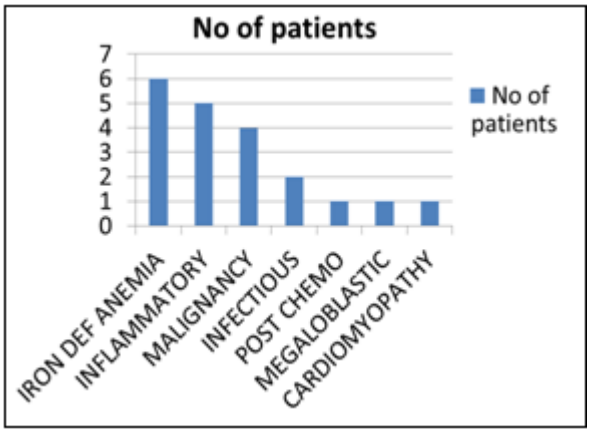


**Figure 7:** Bar diagram showing various etiologies of Reactive cases of thrombocytosis, X-axis- different etiologies; Y-axis-number of patients

**Demography of clonal cases**
The clonal causes of thrombocytosis included different MPNs & MDS. There were 40 cases of which 18 were males and 22 were females. In the clonal group, chronic myeloid leukemia (CML) formed the largest entity and accounted for 26 cases. CMLs presenting with thrombocytosis were seen in any phase. Chronic phase (CP) patients presenting with thrombocytosis accounted for 18 cases. These cases were diagnosed based on morphology in correlation with cytogenetics (Ph chromosome +ve) or molecular studies (BCR-ABL +ve) -results were available in all cases. Two cases met the criteria of blast phase (CML-BP) that is blasts greater than 20% of peripheral blood or bone marrow. Blasts were of myeloid lineage. One case met the criteria of accelerated phase.There were 2 known cases of CML, which had developed secondary myelofibrosis (positive with Masson trichrome stain), one also showed osteosclerosis. There were 2 cases of CML, showing nil hematologic response (NHR) with marked thrombocytosis, following Imatinib therapy. There was also a case of CML on treatment, who had persistent marked thrombocytosis; status post-splenectomy.
There were 4 cases diagnosed as Essential Thrombocytosis based on clinical features, persistent thrombocytosis, morphology and absence of any cause of reactive thrombocytosis. JAK 2 mutation reports were not available for these patients. There were 4 cases diagnosed as Polycythemia Vera; of which 3 showed JAK 2 positivity. They were diagnosed based on clinical and morphological findings along with JAK 2 positivity. There were 2 cases diagnosed as primary myelofibrosis. The diagnosis was made correlating clinical features (massive splenomegaly), leucoerythroblastic blood picture, thrombocytosis with marrow morphology. There were 3 cases diagnosed as MPN U (unclassified) presenting with thrombocytosis. These did not fit the morphologic features of any specific MPN and further cytogenetic/molecular studies were not available. There was one case with thrombocytosis, with dyspoietic features in the marrow. As there was erythroid dyspoiesis, thrombocytosis & isolated Del 5q could not be detected, the patient could not be placed in any specific category.

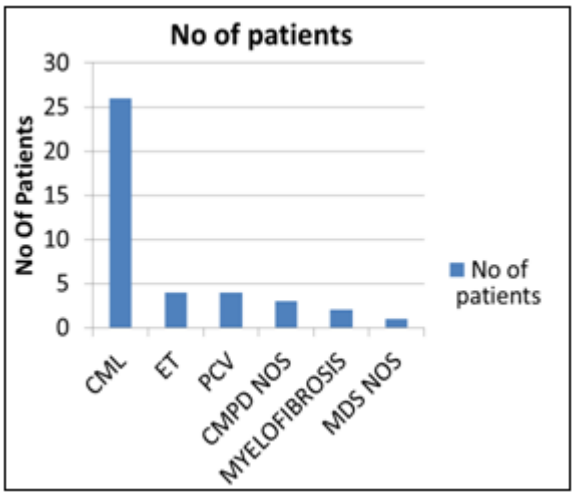


**Figure 9:** Bar diagram showing various etiologies of clonal group

**Platelet Count**
When healthy controls were compared to the reactive and clonal groups, the p-value was <0.001, which meant that platelet count is higher in reactive group and clonal group when compared to control group and is statistically

significant. The p-value was >0.05 when reactive was compared to clonal group, which meant that there is not much difference between reactive and clonal groups regarding the platelet count.

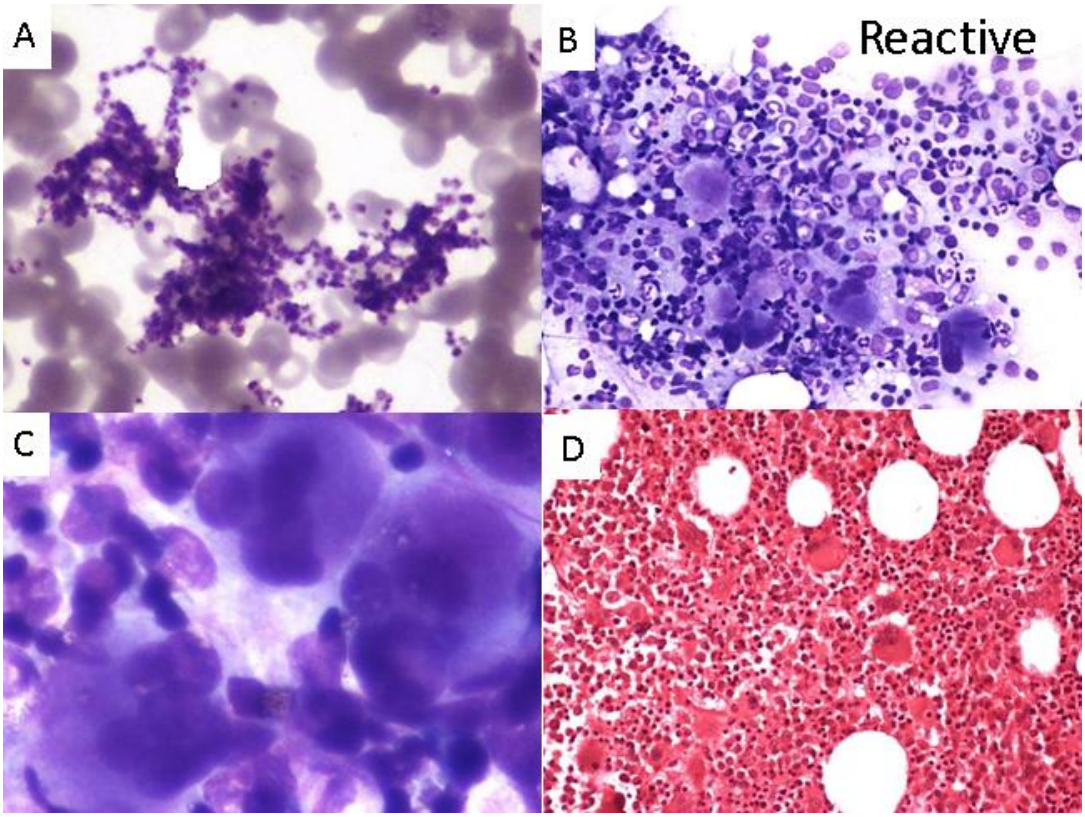


**Figure 16:** Thrombocytosis due to Reactive etiology :A Peripheral smear showing clumping of platelets GiemsaX400, B BMA showing aggregates of megakaryocytes,Giemsa X100, C Megakaryocytes with normal morphology GiemsaX400,D Bone marrow biopsy showing normal distribution of megakaryocytesH/EX100

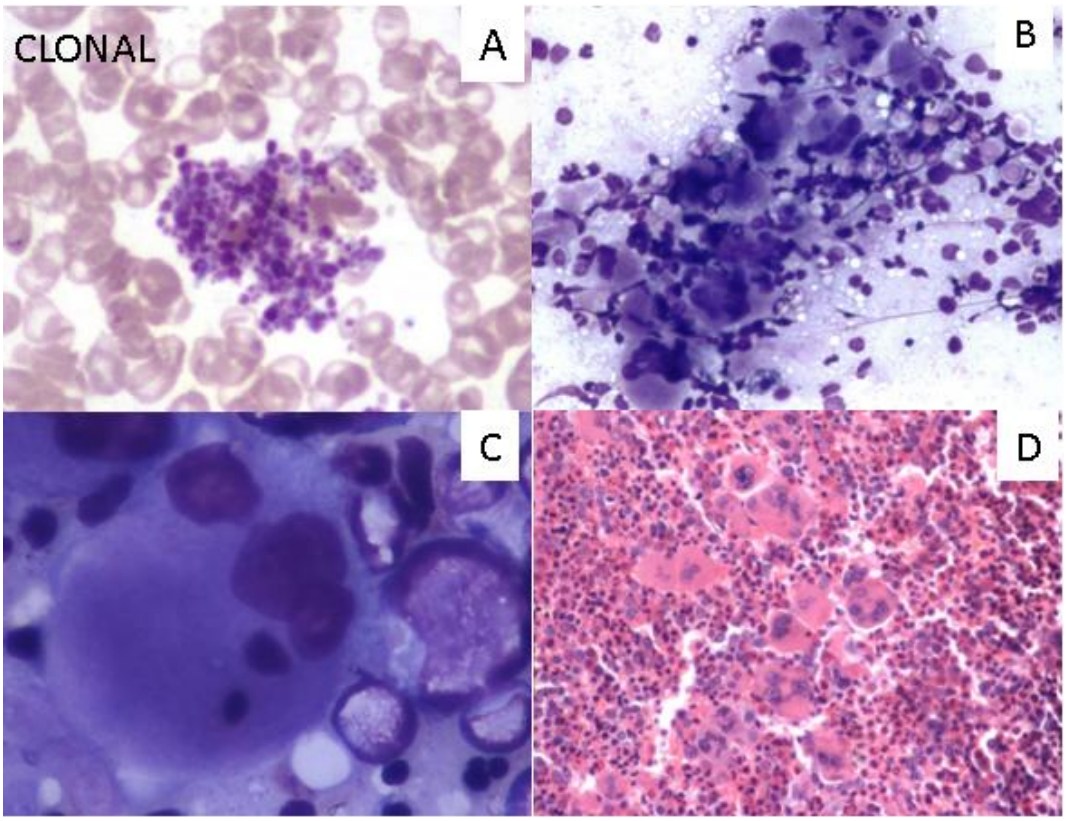


**Figure 17:** Clonal causes of Thrombocytosis-ET A peripheral smear of showing platelet clumps with few giant platelets GiemsaX40, B BMA showing aggregates of megakaryocytes GiemsaX100, C megakaryocytes showing emperipolesisGiemsa X 400, D BM biopsy showing clusters of megakaryocytes H/EX100.

**Mean Platelet Volume (MPV)**

Mean values of MPV of all the three groups were taken and p value was calculated using unpaired T-test. We found that for all the groups Mean Platelet volume values are with in normal limits (Reference value =7.5-11.5fl). However, mean value is higher in clonal group when compared to reactive group which is statistically significant (P<0.01).

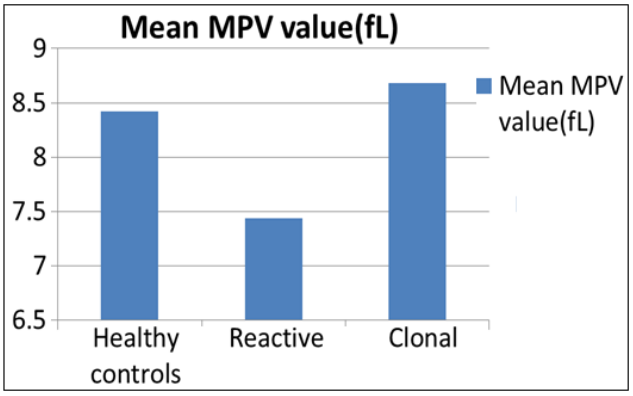


**Figure 10:** Mean values of MPV
P<0.01 between reactive and clonal
<0.01 between reactive and healthy controls

**Platelet Distribution Width (PDW)**

Mean values of Platelet Distribution Width of all the three groups were taken and p value was calculated. Taking normal values from south Indian studies as [94]references (Reference ranges : males9 – 16.56fL, females 8 – 13.28fL), it was found that P<.001 between controls and clonal, reactive and clonal, that is there is significant difference between control group and clonal being higher in later. And also the mean values are found to be higher in clonal group when compared to reactive group. And there is no difference between controls and reactive group. As there is a difference in reference values between males and females, the mean PDW values were studied separately. In males, the values were found to be higher in clonal when compared to controls (P <0.001 between normal and clonal). There was no significant difference between normal versus reactive and Reactive versus Clonal (P>0.05 between normal & reactive and Reactive & Clonal). In females, PDW values are significantly higher in clonal group, when compared to reactive group (P=0.0001 between clonal and reactive). There is not much difference between control group and clonal group (P>0.05 between normal and clonal)

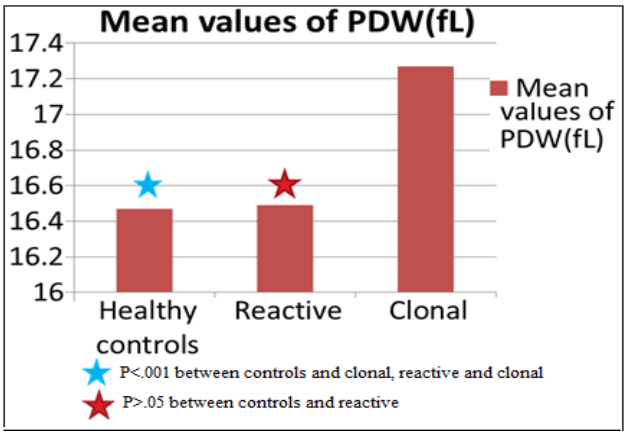


**Figure 11:** Mean PDW values

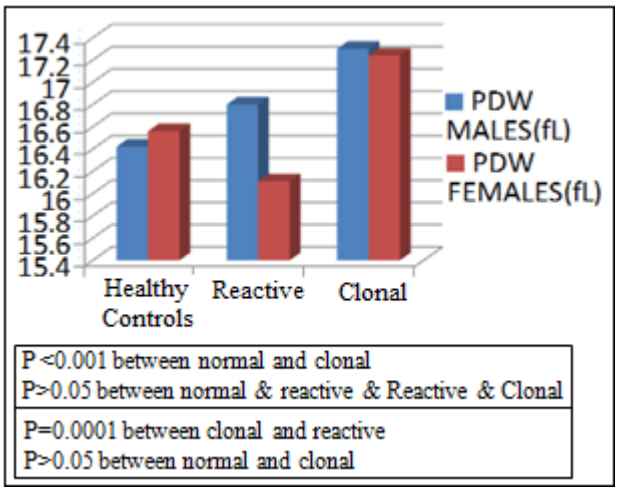


**Figure 12:** Bar diagram showing comparison of mean PDW values in males and females

**Plateletcrit**

Mean values of Plateletcrit of all the three groups were taken and p value was calculated. Reference Range is 0.15 - 0.4 %. The values were higher in the clonal group when compared to reactive group (P<0.01 between reactive and clonal). They were higher in both clonal and reactive group when compared with controls (P<0.01 when controls compared with reactive and clonal).

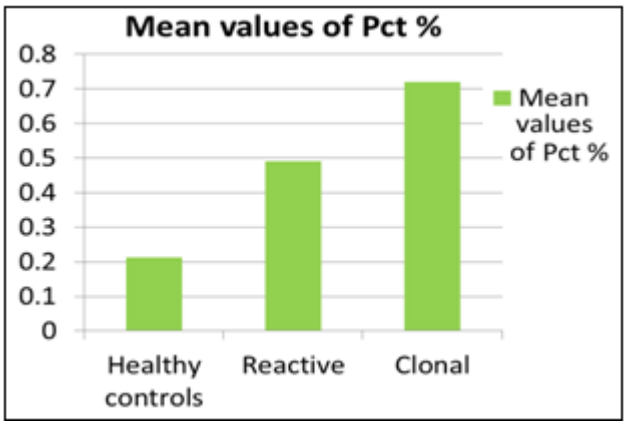


**Figure 13:** Bar diagram showing comparision of mean PCT values

## 4. Discussion

Although routinely available, the platelet indices are generally considered an uninterpretable result. The platelet indices (MPV, PDW) have been found to be clinically useful in distinguishing immune thrombocytopenic purpura (ITP) from thrombocytopenia caused by underproduction of platelets.[10] The platelet indices are probably the most ignored values by clinical laboratories due to the difficulty in standardization, as well as being affected by a range of methodologies. It has been suggested that each laboratory determines its own reference intervals with the equipment used.

The haematological parameters are influenced by various factors like age, ethnicity, diet, genetic and gender differences and hence it is important to define the specific reference values with regards to the age, gender and the region. In our study, we have used reference values from a

south Indian study done in Chennai.[6]

This study also showed that there is significant difference of platelet parameters between these groups. By using these parameters, we could achieve a fairly interesting resolution between reactive and clonal thrombocytosis. This approach is inexpensive and effortless, and requires only the creation of a laboratory database for proper utilization.

In 1997, there was similar study done by Jean-Claude Osselaer.[11] The authors assessed if the combined interpretation of platelet parameters could allow an improved discrimination between patients with reactive and clonal thrombocytosis.

The difference between the earlier study and the present study was that they evaluated all cases with peripheral blood thrombocytosis. Our study included only those cases of thrombocytosis, where corresponding bone marrow examinations were done. Hence the number of cases with reactive causes is less. Also, there is a referral bias. As our hospital has very active Oncology Departments, the number of clonal cases is higher. Similar studies done on platelet indices included more cases from the reactive group than the clonal group.[12 13]

The present study included only 5 children so we could not differentiate these platelet parameters using age. Our centre does not have a Pediatric department.

Comparision of present study with other studies using platelet indices in thrombocytosis

| | Taffazolli etal[2] (2006) | Saeedetal[12] (2009) | Van der Lelie et al[13] (1986) | Present study (2013) |
|---|---|---|---|---|
| Total Number | 146 | 92 | 130 | 60 |
| Reactive | 130 | 80 | 100 | 20 |
| Clonal | 16 | 12 | 30 | 40 |
| Platelet count-lakhs/µLReactive | 6.34 | 5.37 | 6.11 | 6.57 |
| Clonal | 10.10 | 6.25 | 8.52 | 8.46 |
| MPV- (fl ) Reactive | 8.04 | 6.7 | 6.8 | 7.44 |
| Clonal | 9.2 | 7.45 | 7.4 | 8.68 |
| PDW- (fl) Reactive | 9.6 | 7.4 | 16.2 | 16.49 |
| Clonal | 12.85 | 8.7 | 17.8 | 17.27 |
| PCT- (%) Reactive | | | - | 0.49 |
| Clonal | | | - | 0.72 |

## 5. Conclusion

- There was no significant difference of platelet counts between reactive and clonal group (P>.05)
- MPV was significantly high in clonal when compared to reactive group (p<0.01).
- In males, PDW was found to be significantly high in clonal group when compared to normal (p <0.01),
- In females PDW was significantly high in clonal when compared to reactive group (p<0.001).
- Plateletcrit was significantly high in clonal when compared to reactive group (p=0.01).
- To conclude, platelet indices may help us to predict whether thrombocytosis is of reactive or clonal etiology, hence helping us to suggest required ancillary tests.

## References

[1] Jonathan S. Bleeker and William J. Hogan; Thrombocytosis: Diagnostic Evaluation, Thrombotic Risk Stratification, and Risk-BasedManagement Strategies; Thrombosis Volume 2011, Review Article; 1-16
[2] MehriTafazzoli*, Mohammad R. Keramati; Etiology of Thrombocytosis and the Use of Platelet Parameters to Distinguish Between Clonal and Reactive Thrombocytosis;International Journal of Hematology and Oncology. 2006; 16:71-6.
[3] Bashar A. Saeed, Sana M.Taib, Khalid Nafih ; Platelet indices in the differential diagnosis of thrombocytosis ; Annals of the College of Medicine. 2009;5 :1-7.
[4] Van Der Lelie J, Aeg K R Von Dem Borne: platelet volume analysis for differential diagnosis of thrombocytosis, J. CL. Path. 1986; 39:129-133.
[5] **VaniChandrashekar**Plateletcrit as a Screening Tool for Detection of Platelet Quantitative Disorders ; J Hematology: 2013;22-26
[6] **SubhashreeA.R**, ParameswariP.J, The**Reference Intervals** for the Haematological Parameters in Healthy Adult Population of Chennai, Southern India; Journal of Clinical and Diagnostic Research. 2012; 6: 1675-1680
[7] **Khandekar**MM, Khurana AS, Inamdar AK Platelet volume indices in patients with **coronary artery disease and acute myocardial infarction**: an Indian scenario J Clin Path 2006;59:146-9.
[8] **Syed Naveen Naz**, Usman M, Khurshid M **Thrombocytosis** : age dependent aetiology and analysis of platelet indices for differential diagnosis.IJPM 2007 ; 50 :628-33
[9] **Jindal S**, Gupta S, Gupta R, Kakkar A, Singh HV, Gupta K, Singh S. Platelet indices in diabetes mellitus: indicators of **diabetic microvascular complications**. Hematology 2011 Mar;16:86-9.
[10] Platelet distribution curves: interpretation, potentials and limitations ;SysmexXtra Online June 2011.
[11] Osselaer JC, Jacques Jamart, and Jean-Marie Scheiff;Platelet distribution width for differential diagnosis of thrombocytosis; Hematology; Clinical Chemistry43, No. 6, 1997;1072–76
[12] Bashar A. Saeed, Sana M.Taib, Khalid Nafih ; Platelet indices in the differential diagnosis of thrombocytosis ; Annals of the College of Medicine.2009;5:1-7.
[13] Van Der LelieJ, Aeg K R Von Dem Borne: platelet volume analysis for differential diagnosis of thrombocytosis, J. CL. Path. 1986; 39:129-133