

Research Article

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Plateletcrit in differenting quantitative abnormalities of platelet

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Abstract

Background: Complete hemogram is routine blood investigation requested by clinician in day to day practice as it's the basic step of investigation which provides instant overview of hematopoietic system at a particular time. Precise and faster results are provided by automated haematology analysers. Among the several parameters, platelet indices are gaining importance as its clinically valuable biomarker. **Objective:** To determine normal reference range for plateletcrit which could differentiate quantitative abnormalities of platelets. **Method:** We studied platelet parameters in 50 with normal platelet count (Group A), 100 were thrombocytopenia (Group B) and 106 were thrombocytosis (Group C). The blood samples were analysed using Sysmex XT 1800i. The platelet count and platelet indices - plateletcrit (PCT) values were recorded. The results were statistically analyzed. **Results:** The mean age of Group A is 39±2.08. The mean age of Group B and Group C is 42.7±1.5 and 42.1±1.56 respectively. The mean platelet count and platelettric was analyzed in all three groups, at various age intervals of < 20 years, 21- 40 years, 41-60 years and > 60 years. No statistically significant difference noted within groups (A, B & C), in age interval and within gender. A significant positive correlation was noted in all groups between platelet count and plateletrit. The reference range for plateletrit was identified (0.16 - 0.36%) by receiver operating characteristics curve (ROC) and area under curve (AUC). **Conclusion:** Establishing the local reference ranges for platelet parameters, with the uniformity in analysing method, aids the clinician for appropriate patient management.

Keywords: Plateletcrit, Platelet count, Reference range, Thrombocytopenia, Thrombocytosis.

INTRODUCTION

Platelets are the dynamic blood particles which is the cytoplasmic fragments of megakaryocytes. Hemostasis is the primary function. Platelet also has role in inflammatory process, microbial host defence, wound healing, angiogenesis and remodelling [1]. With the advent of automated haematology analyzers, not only that it provides rapid and accurate measurement of platelet count but also the other indices such as Mean platelet volume, Platelet volume distribution width, plateletcrit and platelet large cell ratio in the given blood sample without bringing extra cost. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of settings [2].

Plateletcrit reflects prothrombotic state in specific population. It is similar to haematocrit for erythrocytes. It is one of the biomarkers of platelet activation [3]. Ugur *et al* [4] reported significance of plateletcrit values to predict long term cardiovascular mortality. Akpinar *et al* [5] also has reported that increase in plateletcrit is significantly associated with saphenous venous graft disease. But the reference ranges for plateletcrit with which the patient's results should be interpreted are not known. The reference ranges indicated by manufacturers may not be suitable for every laboratories [6]. Therefore, local reference ranges for platelet indices should be determined. Therefore, the aim of the study is to determine normal reference range for plateletcrit which could differentiate quantitative abnormalities of platelets.

MATERIALS AND METHOD:

This is a prospective cross-sectional study. We studied 256 EDTA anticoagulated blood samples, for platelet parameters. The samples were collected by venepuncture under sterile aseptic technique and was processed within 2 hours of collection, using Sysmex XT 1800i. Adults of both genders were included in the study. Patients who are on antiplatelet drugs, anti-inflammatory drugs causing thrombocytopenia (aspirin,

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Dr. Shivashekar Ganapathy Professor, Department of Pathology, SRM Medical College Hospital & Research Centre, Potheri, Chennai-603203, Tamil Nadu, India ibuprofen) and clotted blood sample were excluded from the study.

Peripheral smears were stained with Romanowsky stain (Leishman stain), according to institutional standardized operating procedure. The smears were screened to rule out pseudo-thrombocytopenia. 256 cases were grouped into three categories. 50 cases with normal platelet count (1.5 - 4.5 lakhs per cu.mm) as Group A. 100 cases Group B with thrombocytopenia (less than 1.5 lakhs per cu.mm) and 106 cases in Group C with thrombocytosis (more than 4.5 lakhs per cu.mm).

Statistical analysis was done using SPSS version 19. Descriptive statistics of mean, standard error, range, frequency and percentage were used to describe the data. Inferential statistics of unpaired 't' test, ANOVA, Pearson correlation were used to analyze the data. Receiver operating characteristic curves (ROC) used to identify the reference range for plateletcrit and sensitivity, specificity, positive and negative predictive value were estimated.

RESULTS

Demographics:

The mean age of Group A is 39 ± 2.08 with standard deviation of 14.72. The age ranged from 15 to 80years. The mean age of Group B and Group C is 42.7 ± 1.5 and 42.1 ± 1.56 respectively. The standard deviation is 15.18

Table 1: Difference in mean age between male and female

and 16.15 in Group B and Group C respectively. The age ranged from 18 to 72 years in Group B and 20 to 83 years in Group C. The mean age among 35 males is 37.94 ± 2.53 and in 15 females is 41.47 ± 3.69 in Group A. The mean age among 71 males in Group B and 49 in Group C is 42.41 ± 1.72 and 44.54 ± 2.38 respectively. The mean age among 29 females in Group B and 57 in Group C is 43.52 ± 3.16 and 40.02 ± 2.06 respectively (Table 1).

Platelet count:

Statistical analysis with ANOVA test was done to study, if any difference in mean platelet count was present in cases with normal, low and high platelet counts, in various age groups of less than 20 years, 21- 40 years, 41-60 years and more than 60 years (Table 2). The difference in mean platelet count was studied in cases with normal, low and high platelet counts, at various age interval were found to have no statistical significance. The mean platelet counts in among males in Group A, B and C is 229314.29±8628.74, 96690.14±4632.69 and 546320±13768.72 respectively. Among females, the mean platelet count in Group A is 255466.67±16112.07, in Group B is 102827.59±6548.99 and in Group C is 525178.57±9484.06. Any variation of the mean platelet count with respect to gender was analyzed by using unpaired't' test. No statistically significant difference noted within groups (A, B & C), among both the gender, with p value of 0.127, 0.465 and 0.201 respectively.

Group	Male	Female	T test	P value
A	37.94 ± 2.53	41.47 ± 3.69	-0.772	0.444
В	42.41 ± 1.72	43.52 ± 3.16	-0.330	0.742
С	44.54 ± 2.38	40.02 ± 2.06	1.446	0.151

Table 2: Difference in mean Platelet count between age groups

Group	<20	21 - 40	41 - 60	>60	ANOVA	P value
А	228000.00 ±	239652.17 ±	236812.50 ±	236166.67 ±	0.058	0.981
	21260.29	12431.41	12765.26	27918.23		
В	72833.33 ±	98093.02 ± 6091.12	98105.26 ± 5338.84	112615.38 ±	1.546	0.208
	23415.69			8836.51		
С	527750.00 ±	526520.00 ±	544682.93 ±	541545.45 ±	0.370	0.775
	28558.06	9122.76	17139.86	21258.74		

Table 3: Difference in mean Plateletcrit between age groups

Group	<20	21 - 40	41 - 60	>60	ANOVA	P value
A	0.23 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.02	0.358	0.783
В	0.08 ± 0.03	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	1.836	0.146
С	0.49 ± 0.03	0.49 ± 0.01	0.50 ± 0.01	0.49 ± 0.02	0.049	0.985

Table 4: Comparison of Plateletcrit with other published studies

Study	Number of cases	Instrument	Population	Reference range of PCT%	Pct %
Vani Chandra sekar 2013	206	Advia 2120	Indian	0.20 - 0.36	0.09 thrombocytopenia 0.23normal 0.50 thrombocytosis
Giovanetti 2011	306	Bayer Advia 120	Italian	0.17 - 0.38	0.24 males 0.28 females
Wiwanikit 2004	215	Technicon H*3	Thai	0.23-0.24	0.24 males
Adibi 2007	19993	Technicon H*2	Iranian	0.13-0.32	0.22 males and females
Botma 2012	60	Sysmex XE 2100	South african	0.19-0.40	0.29 males 0.23 females
Present study	256	Sysmex XT 1800i	South Indian	0.165-0.365	0.10±0.01 thrombocytopenia 0.23±0.01for males and 0.26 ±0.01for females with normal platelet count 0.50±0.01 thrombocytosis

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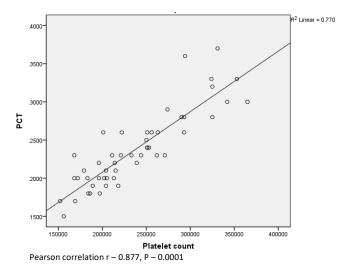
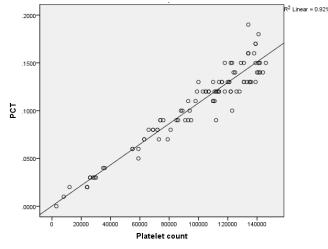


Figure 1: Correlation between platelet count and plateletcrit in Group A



Pearson correlation r – 0.960, P – 0.0001

Figure 2: Correlation between platelet count and plateletcrit in Group B

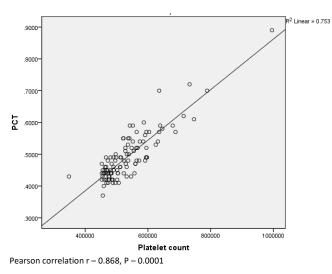


Figure 3: Correlation between platelet count and plateletcrit in Group C

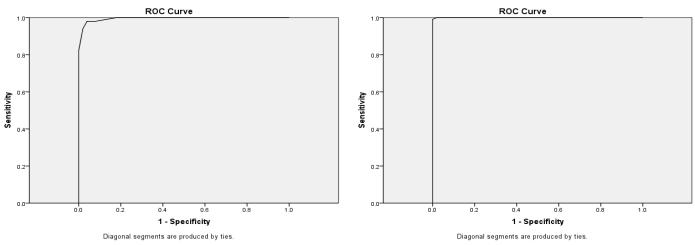


Figure 4: Group B: At cut off value 0.165, the Area under the curve – 0.995; Sensitivity – 98%, Specificity –96%

Figure 5: Group C: At cut off value 0.365, Area under the curve – 1.000; Sensitivity – 100%, Specificity – 99.8%

Plateletcrit:

The mean plateletcrit in different age groups are tabulated (Table 3). The mean plateletcrit in different age intervals were noted to be statistically insignificant. Among males the mean plateletcrit value in Group A is 0.23 ± 0.01 , Group B is 0.10 ± 0.01 and Group C is 0.50 ± 0.01 . The mean plateletcrit values among females in Group A is 0.26 ± 0.01 , Group B is 0.11 ± 0.01 and Group C is 0.49 ± 0.01 . No statistically significant difference noted in plateletcrit among males and females and their p value is 0.074, 0.309 and 0.285 in Group A, B and C.

Correlation between Platelet count and Plateletcrit:

The relation between platelet count and plateletcrit was analyzed using Pearson correlation test (Figure 1 - 3). A strong significant positive correlation was noted in all groups whereas maximum significant positive correlation was noted in thrombocytopenic group (Group B).

Reference range:

In this study, the normal range for platelet count was 152000 to 365000 per cubic millimetre. The receiver operating characteristic curves (ROC) were plotted and area under curve (AUC) was calculated to identify the reference range for plateletcrit (Figure 4-5). At the cutoff value of 0.165, area under the curve is 0.995 and the sensitivity is 98%, specificity is 96%, positive predictive value is 99% and negative predictive value is 92.5% for detection of thrombocytopenia. At the cutoff value of 0.365, area under the curve is 1.00 and the sensitivity is 100%, specificity is 98%, positive predictive value is 99.1% and negative predictive value is 100% for detection of thrombocytosis. The reference range of plateletcrit is 0.165 - 0.365 in the present study population.

DISCUSSION

For decades, bone marrow examination remained standard method for evaluating quantitative abnormalities of platelet. But it is invasive and time-consuming procedure with complication of bleeding and infection. Serological and molecular analysis for differentiating various causes of quantitative abnormalities of platelets is not economical and available at all laboratory centres. In manual method of evaluation of platelets, there is a delay between blood sample collection and smear preparation. The platelet morphology is altered due to its increased adhesiveness and flattening effect on smear, platelets show pseudo increase in its diameter. Platelet indices obtained through automated analyser can avoid observer bias [7].

Plateletcrit is an expression of a percentage that reflects the volume occupied by platelets in blood. It is a quantitative test for variations in platelet count. It is directly related to platelet count and size of the platelets. It can differentiate reactive thrombocytosis from myeloproliferative disorder [7-11]. It helps to assess whether cardiopulmonary bypass patients require platelet transfusion [9].

Reference ranges when well defined and accurate is valuable in the interpretation of results & appropriate patient management [12]. Thrombocytic function is assessed using the platelet indices. Certain diseases such as Alzheimers disease, diabetes, inflammatory bowel disease and glomerular diseases [13]. Establishing local reference ranges is essential as different studies have reported different reference ranges.

Demography:

The participant's age ranged from 15 to 83 years. Though there was slight difference in the mean age between the genders, in cases with normal platelet count (Group A), it was not statistically significant (p value is 0.44). Similarly, no significant difference noted in thrombocytopenic (Group B) and thrombocytosis patients (Group C). Therefore, age did not have a role in the gender reference ranges

comparison. Giacomini *et al* [13] observed that platelet count and plateletcrit decrease from childhood but remain steady afterwards.

Platelet count:

Pekelharing *et al* [14] found significant difference between male and female with p value less than 0.0001. The difference can possibly be ascribed to the biological variation of hormonal status in gender or the effect of menstrual cycle on the haemostatic mechanism. In the present study, no significant difference is observed with the average platelet count and plateletcrit in all groups, with respect to age and gender. Butkiewicz *et al* [15] discovered that thrombopoietin levels are lower in females than males. The reference range of platelet count in the study population was 152000 – 365000 per cubic millimetre with the mean platelet count of 237 X 10⁹/L. In a study in Indian population among blood donors, north eastern India had the mean platelet count of 132 X 10^{9} /L (Range 71 - 267) and in south Indian population was 252 X 10^{9} /L (Range160-478) [16]. The mean platelet count and reference range is in line with the other studies.

Correlation of Platelet count with Plateletcrit:

Pearson correlation test showed strong positive correlation between platelet count and plateletcrit, in the present study. It infers that plateletcrit is directly proportional to platelet count in all groups. The similar observation is noted by various authors [13, 17].

Plateletcrit

The mean plateletcrit among males is $0.23{\pm}0.01\%$ and $0.26{\pm}0.01\%$ in females in this study. It is similar to Giovanetti TV [18] (0.24%), Chandrasekhar V [19] (0.23%), Wiwanikit [17] (0.24%), and Adibi et al [20] (0.22%) (Table 4). However, plateletcrit value of the present study is higher than observed by Naina HV et al (0.17 in North east India and 0.19 in South India) [20] and Beyan et al (0.17% in Iranian population) [21]. The reference range of plateletcrit 0.165 - 0.365% in the present study population was helpful in distinguishing patients with normal platelet count, thrombocytopenia and thrombocytosis. The sensitivity and specificity for detection of thrombocytopenia is 96% and 98%. For thrombocytosis, the sensitivity is 100% and specificity is 98%. Chandrasekar V et al [19], noted significant overlap of about 46.3% with normal platelet count and low plateletcrit values, due to differences in mean platelet volume. Whereas no such significant overlap was noted with thrombocytopenia or thrombocytosis in this study. In few cases of thrombocytopenia, output values for platelet indices was not generated due to deviation of normal bell-shaped curve in the histogram. Similar limitation was noted by Tocantis et al [22] & Niethermmer AG et al [23].

Factors influencing variation of the reference range results of present study, in comparison with other studies includes non-uniformity in the automated analyzers, usage of different reagents, difference in the principle of measurement technique and the calibration of the haematology analyser. The geographical variation of population under study also influences the difference in the reference range. The method of blood sample collection, the degree of accuracy of filling and mixing the vacutainers may also influence variation of platelet parameters between studies.

CONCLUSION

The reference range of plateletcrit for detection of thrombocytopenia and thrombocytosis, obtained in the study, is comparable well with other studies in the literatures. The minor variations noted with other studies is not ignorable but in turn signifies establishing reference range for each laboratory; by reducing the factors influencing the variation like instrumentation and sample collection method. These established local reference range will enable the laboratory to effectively use the automated analyser and provide better service to clinician. Clinicians can perform critical evaluation of platelet count with its indices which

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could narrow down the differential diagnosis. Further large, multicentre prospective studies with large population, concurrently collecting data from different ethnicities and genders are recommended for exploring the utility of platelet parameters in various clinical conditions.

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