Assessment of Platelet Count in Normal Kashmiri Population

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ABSTRACT

Introduction: Macrothrombocytopenia, a combination of thrombocytopenia and giant platelets, can occur in a number of diseases, including chronic immune thrombocytopenia purpura and inherited giant platelet disorders. It is present in at least 12 inherited syndromes as well as in some acquired immune-mediated and hematological disorders. Study aimed to evaluate the platelet count in normal Kashmiri population,using hematology analyser sysmex XT-2000i in comparison with manual platelet counting(Neubauer chamber) recommended by the international committee for standardization in Hematology.

Material and Methods: In present study, 500 samples of blood were collected from apparently Normal kashmiri Population and were processed by automated analyzer (Sysmex XT-2000i) and Manual Platelet Counting (Neubauer chamber). Multiple comparisons were done between the Automated and Manual method.

Results: The result demonstrated variation in platelet count between automated analyzer and Manual Platelet Counting.The mean value of platelet count estimated by automated analyzer was found to be 126.40 x 10^3/µl (Range: 47.0-394.0 x 10^3/µl) while the mean value of platelet count estimated by manual method was 139.06 x 10^3/µl (Range: 60.0-390.0 x 10^3/µl) and was found to be statistically significant ($p<0.0001$).Similarly, the mean value of platelet count estimated by automated analyzer in males and females was found to be 125.64 x 10^3/µl (Range: 47-394 x 10^3/µl) and 152.93 x 10^3/µl (Range: 84-223 x 10^3/µ) respectively. During analysis it was found that platelet count by manual method is higher as compared to the automated method in our laboratory.

Conclusion: The study highlights the differences in platelet count in our population using automated and manual method. Higher platelet count by manual method in our population may be because of large platelet size which analyzer's are not able to count and therefore the results should be carefully interpreted.

Keywords: Platelet count, Automation, Manual, Giant platelets.

INTRODUCTION

Platelet count is important diagnostic tool so it necessary to count the platelets accurately. Before the widespread use of automated counters, Manual platelet count was done. The manual count is the oldest means of counting platelets and, remarkably, is still used as the gold standard international reference method.[1] Recently, a new immunoplatelet counting procedure has been proposed as the new international reference method. Although modern impedance counters are rapid, precise and reproducible, they tend to overestimate the platelet count in samples that contain cellular debris, e.g. thalassaemia, thrombocytopenic purpura (TTP). It is necessary to count as the transfusion threshold for platelets is now set at $10x10^9$/l.[2-5] Recent analysers work on optical counting methods.[6-7] New methods increase the accuracy of the count as both normal and large-sized platelets are easily discriminated from noise and other cell populations. Recent evidence confirms that optical counters agree more closely with a newly proposed platelet counting reference procedure. In the latter method, platelets are identified via flow cytometric analysis of samples that have been incubated with a monoclonal antibody (e.g. anti- CD61).[8] Due to superiority of the RBC ratio to the manual count, The method can also be transferred to recent analysers like Abbott Cell Dyn 4000.[9-10] It is now possible that platelet transfusion threshold could be reduced down to as low as $5x10^9$/l in severe thrombocytopenia by accurate platelet counting. Study aimed to evaluate the platelet count in normal Kashmiri population, using hematology analyser sysmex XT-2000i in comparison with manual platelet counting (Neubauer chamber) recommended by the international committee for standardization in Hematology.

MATERIAL AND METHODS

Collection of Blood samples

A total of 500 blood samples from normal Kashmiri population were used to conduct the study. 5ml of venous blood was obtained through vein puncture in an EDTA coated (0.5M, pH-8.0) sterilized plastic vials. The vials were properly labeled according to a specially designed coding system. The coding system was developed so as to prevent possible mixing of the sample vials and for easy retrieval of the required blood sample vial. All blood samples were analyzed within 4 hours after phlebotomy. Healthy donors were age 20 or older.

Informed consent was taken and the study was cleared by SKIMS ethical clearance committee.

Following are inclusion and exclusion criteria which were adopted for the study.

Inclusion criteria: Normal Kashmiri population

Exclusion criteria: History of chronic illness.

Automated platelet count

Automated platelet count was done using Sysmex XT-2000i. This is commonly used in laboratories with high sample load as in our setting.

Manual Platelet Counting

Venous blood sample was collected into a dry plastic syringe.

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The blood and anticoagulant was mixed gently, to avoid frothing, without any delay. Whole blood was diluted with a 1% ammonium oxalate solution. The erythrocytes were lysed by diluent while the leukocytes, platelets and reticulocyte remained intact. The standard dilution for platelet counts was 1 in 20. The sample was incubated for sometime and mounted on a haemocytometer. The cells were allowed to settle and then counted in a specific area of the haemocytometer chamber under the microscope. The number of platelets was calculated per microlitre (x 10^9/L) of blood.

Materials

1. Blood samples (EDTA): control and test.
2. Improved Neubauer counting chamber.
3. Adjustable pipettes
4. 1% Ammonium oxalate solution for dilution.
5. Test tubes
6. Test tube rack
7. Glass capillary tubes
8. Petri dish with wet filter paper
9. Mechanical mixer

Microscope

Ordinary light microscope. Condenser was racked well down to provide enough contrast to 'see' the platelets.

Method

1. Test tubes were labelled as 'control' or 'test' and a 1.9ml of ammonium oxalate was added to each tube.
2. 100µL of well mixed test or control blood sample was added to the appropriate tube.
3 Diluted samples were mixed by inversion.
4. Mirrored surface of the counting chamber was cleaned gently.
5. Counting chamber was placed on the stage of the microscope and with the 10X objective the surface was checked for scratches.
6. Glass cover slip was cleaned gently.
7. Moisture (water) over each of the raised transverse platforms of the counting chamber was wiped.
8. Glass cover slip was put into position along the platforms forming a sealed chamber over the engraved counting grids.
9. Capillary tube 2/3 to ¾ full of well mixed (gently - no air bubbles) diluted sample was filled.
10. Capillary tube was touched to the edge of the loading groove on one side of the counting chamber and we allowed the diluted sample to fill the chamber.
11. The counting chamber was placed in the damp chamber for 20 minutes to allow the platelets to settle.
12. Bottom of the chamber was wiped carefully to remove excess moisture from the moist box and place chamber on the microscope stage.
13. Using the 10X objective, we focused on the engraved counting area to look for the central 1mm square.
14. Then we changed to 40X objective to focus on one of the 25 squares contained in the central 1mm square.
15. The number of platelets in each of 16 squares in zig zag manner were counted including the platelets over or attached to either of two sides.
16. Steps 14 and 15 were repeated four more times to count the platelets in a total of 5/25 squares.
17. The total number of platelets per litre were calculated for the control and test samples using the general calculations for manual cell counting.

Calculations

The hemacytometer counting chamber (improved Neubauer) was used (figure-1)

The dilution of blood for platelet counts was 1 in 20; therefore the dilution factor was 20. The volume of diluted blood used was based on the area and depth of the counting area. The area counted was 5mm and the depth was 0.1mm, therefore the volume factor was 0.5mm.

The formula used for calculating the cell count was:

$$\text{Cells/mm}^3 = \frac{\text{No. of Platelets x dilution factor}}{\text{Area counted x depth of fluid}}$$

Where dilution was 1/20

Area counted was 80/120 = 1/5 sq.mm

Since cells were counted in 5 bigger squares and each square was further divided into 16 small squares.

Area of each square was = 1/400 sq.mm

Hence area of 80 such squares = 80/400 = 1/5 sq.mm

STATISTICAL ANALYSIS

Microsoft office 2007 was used for statistical analysis. Descriptive statistics like mean and percentage were used for data interpretation.

RESULTS

Platelets play a key role in both homeostasis and thrombosis. It is important to measure platelets accurately for identifying patients with either platelet dysfunction and monitoring modern antiplatelet therapy.

In present study, 500 samples of blood were collected from apparently Normal kashmiri Population and were processed by automated analyzer (Sysmex XT-2000i) and Manual Platelet Counting (Neubauer chamber). Multiple comparisons were done between the Automated and Manual method.

The result demonstrated variation in platelet count between automated analyzer and Manual Platelet Counting.

Age wise variation of Platelet count using Automated platelet count analyser and Manual method is given in Table 1.

The mean value of platelet count estimated by automated analyzer was found to be 126.40 x 10^3/µl (Range: 47.0-394.0 x 10^3/µl) while the mean value of platelet count estimated by manual method was 139.06 x 10^3/µl (Range: 60.0-390.0 x 10^3/µl) and was found to be statistically significant ($p<0.0001$) (Table 2).

Similarly, the mean value of platelet count estimated by automated analyzer in males and females was found to be 125.64 x 10^3/µl (Range: 47-394 x 10^3/µl) and 152.93 x 10^3/µl (Range: 84-223 x 10^3/µ) respectively. While the mean value of platelet count estimated by manual platelet counting in males and females was found to be 138.42 x 10^3/µl (Range: 60-390 x 10^3/µl) and 161.43 x 10^3/ µl (Range: 95-230 x 10^3/µl) respectively and was to be found statistically significant ($p<0.0001$).

While studying 124 samples (24.8%) of the individuals falling in the age group of 15-25years, the mean value of platelet count by using Automated method was 139.07 x 10^3/µl (Range: 47-247 x 10^3/µl) and by using manual method was 151.43 x 10^3/µl (Range: 60-370 x 10^3/µl). In the age group of 25-35, there were

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Auto Platelet count	15-25	124	139.07	44.024	3.953	131.25	146.90	47	247
	25-35	205	124.85	48.287	3.373	118.20	131.50	54	394
	35-45	140	120.98	45.074	3.809	113.45	128.51	47	286
	45-55	26	105.46	40.490	7.941	89.11	121.82	53	213
	55-65	5	136.40	65.137	29.130	55.52	217.28	56	200
	Total	500	126.40	46.755	2.091	122.29	130.51	47	394
Manual platelet count	15-25	124	151.43	44.425	3.989	143.53	159.32	60	370
	25-35	205	137.56	44.355	3.098	131.45	143.66	65	390
	35-45	140	133.41	41.686	3.523	126.44	140.37	60	280
	45-55	26	120.92	38.974	7.643	105.18	136.66	73	220
	55-65	5	147.00	58.052	25.962	74.92	219.08	70	200
	Total	500	139.06	44.087	1.972	135.19	142.94	60	390

Table-1: Age wise variation of platelet count
This table shows mean value of platelet count highest by mean value platelet counting than automated counting in all age groups

		Sum of Squares	df	Mean Square	F	Sig.
Platelet count	Between Groups	36420.744	4	9105.186	4.274	.002
	Within Groups	1054409.256	495	2130.120		
	Total	1090830.000	499			
manual platelet count	Between Groups	32771.361	4	8192.840	4.328	.002
	Within Groups	937096.591	495	1893.124		
	Total	969867.952	499			

Table-2: ANOVA platelet count between and within groups

	Method	N	Mean	Std. Deviation	Std. Error Mean	p-value
Platelet count	Auto-Platelet count	500	126.40	46.755	2.091	<0.0001*
	Manual count	500	139.06	44.087	1.972	

Table-3: Variation of platelet count by Auto-platelet count and Manual count

205 cases (41%), while analysing these cases the mean value of Automated platelet count was 124.85 x 10^3 /µl (Range: 54-394 x 10^3/µl) and the mean value by manual method was found 137.56 x 10^3/µl (Range: 65-390 x 10^3/µl). While in the age group of 35-45, analysis of 140 blood samples (28%) was done. During the analysis the mean value of Automated and Manual platelet count was found 120.98 x 10^3/µl (Range: 47-286 x 10^3/µl) and 133.41 x 10^3/µl (Range: 60-280 x 10^3/µl) respectively. 26 samples (5.2%) of the donors of the age group of 45-55yrs were studied. The mean value of platelet count was found 105.46 x 10^3/µl (Range: 53-213 x 10^3/µl) and 120.92 x 10^3/µl (Range: 73-220 x 10^3/µl) by using Auto and manual methods respectively. Only five samples (1%) of males of the age 55-65yrs were studied. The mean value of Auto platelet count was found 136.40 x 10^3/µl (Range: 56-200 x10^3/µl) and mean value by manual method was found 147 x 10^3/µl (Range: 70-200 x 10^3/µl) and was found to be statistically significant ($p<0.05$).

While studying all the 500 samples, the mean value of Automated platelet count was found 126.4 x 10^3/µl. When the samples were analysed manually the mean value of Manual platelet count was found 139.06 x 10^3/µl and was found to be statistically significant ($p<0.05$) (Table-3).

Variation of platelet count by Automated and manual methods (Table 2)

The samples were analysed for platelet count by using both Auto and manual methods. The maximum count (139.07 and 151.43 x 10^3/µl) was seen in the age group of 15-25yrs, while as, minimum count (105.46 and 120.92 x 10^3/µl) was found in the individuals of the age group of 45-55yrs by using automated and manual methods of platelet count respectively.

The same blood samples were also used for Manual platelet count. The study shows that females have comparatively higher platelet than males as estimated by Manual platelet method. The Mean value of Manual platelet count was found 138.42 x 10^3/µl in males and 161.43 x 10^3/µl in females and was found to be statistically significant ($p<0.05$).

During our study males were more in number than females and the mean value of platelet count was found highest in females than males by both automated platelet counting and manual platelet counting analysis.

CONCLUSION

The study highlights the differences in platelet count in our population by using automated and manual methods. During analysis it was found that platelet count by manual method is higher as compared to the automated method in our laboratory. The possible reason in our population could be the large platelet size (Giant platelets, size 10-20µm), which the analysers are not able to count and therefore the results should be carefully interpreted.

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