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RESEARCH ARTICLE

ESTIMATION AND COMPARISON OF PLATELET COUNT: AUTOMATED ANALYZERVS MANUAL PERIPHERAL SMEAR METHOD

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Abstract

Accurately assessing platelet counts holds paramount importance in the realm of diagnostics and treatment. The adoption of automated methods within the diagnostic landscape is steadily growing, attributed to their multifaceted benefits. Nonetheless, automated analyzers sometimes yield erroneous results, particularly in scenarios involving particles of comparable sizes or instances of light scattering. These encompass fragmented red blood cells (RBCs), microcytic RBCs, and the presence of oversized platelets or platelet clusters. In response, the manual estimation of platelet counts using Leishman's stain validation gains significance.

Method: this study encompassed the collection of blood samples from a diverse pool of 100 patients, including both inpatients and outpatients, spanning the period from 1st February 2023 to 31st July 2023. These samples, procured from GMC Doda and its affiliated hospital, were collected in ethylenediaminetetraacetic acid (EDTA) tubes. The manual platelet count was done, followed by a comprehensive comparative analysis with the counts obtained through automated platelet analysis.

Results: the outcomes unveiled a nuanced trend, with manual slide-derived results slightly surpassing those generated by automated analyzers.

Conclusion: This study concluded a discernible correlation between automated and manual methodologies for platelet count assessment. Nevertheless, in instances marked by exceedingly high or low platelet counts, the manual method holds its ground, ensuring precision by circumventing concerns like platelet clumping or uneven distribution.

Introduction:-

Accurate estimation of platelet counts stands as an indispensable pillar within the dynamic realm of diagnostics and therapeutic interventions. These non-nucleated, discoid entities, measuring 1 to 3µ in diameter, come into existence through the intricate process of megakaryocyte cytoplasmic fragmentation within the confines of the bone marrow[1]. Their pivotal roles extend to the orchestration of homeostasis and thrombosis[2]. The delicate equilibrium is signified by the physiological platelet count range of 1,50,000 to 4,50,000/mm³, observed within healthy individuals[3].

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With the emergence of dengue fever in the past few years, platelet count assessment has ascended to the echelons of routine diagnostics across pathology laboratories. It proves paramount for individuals undergoing chemotherapy and assumes a central role in management of pregnancy-induced hypertension, malaria, bacterial sepsis, and leukemia[4]. The compass of its importance extends further, enfolding the diagnostics and strategic management of hemorrhagic disorders. The foundational techniques for platelet estimation include manual quantification through specialized counting chambers, meticulous examination of peripheral smears, and the contemporary domain of automated cell counters. These methodologies, each adorned with distinct attributes and constraints, coalesce to decipher the enigma of platelet counts, contributing to the broader narrative of medical diagnosis and intervention.

Furthermore, the International Council for Standardization in Hematology (ICSH) and the International Society for Laboratory Hematology (ISLH) advocate immunoplatelet counting as the reference method for calibrating automated hematology analyzers[5]. This reference methodology necessitates the deployment of flow cytometers and the expertise of trained technicians. Although automated hematology analyzers typically yield precise and accurate platelet count measurements, challenges arise when dealing with exceedingly low counts or encountering interference from non-platelet particles or platelet anomalies[6].

A traditional yet reliable method, involving the enumeration of platelets within peripheral smears through manual assessment, has been in practice for an extended duration[7]. This approach entails the multiplication of the average platelet count within oil immersion fields by either 20,000 or 15,000 to yield an estimated platelet count per µl. Nonetheless, this method harbors inherent limitations.

In light of these intricacies, the fundamental objective of this study is to rigorously evaluate the diagnostic accuracy of automated analyzers vis-à-vis the time-honored manual approach in the estimation of platelet counts.With the help of this study, we aspire to unravel the complexities underpinning platelet quantification, ultimately enhancing our understanding of this cornerstone diagnostic parameter.

Aims And Objectives:-

The primary aim of this study is to meticulously evaluate, compare, and establish correlations between platelet count estimations derived through the manual slide method and the automated analyzer technique.

Materials And Methods:-

This investigation was carried out at the Government Medical College and its affiliated hospital in Doda. This was a prospective cross-sectional study conducted over the period spanning from 1st February 2023 to 31st July 2023. A total of 100 patient blood samples were meticulously collected, spanning a diverse range of ages. These samples were managed with utmost confidentiality and labeled comprehensively with pertinent details including the patient's name, age, sex, and a unique serial number.

Inclusion Criteria:

The study included both inpatients and outpatients referred to the Department of Pathology at Government Medical College Doda from various clinical departments of the hospital and its affiliated healthcare institutions. All individuals, regardless of age and sex, undergoing routine complete blood count assessments were eligible for inclusion.

Exclusion Criteria:

Blood samples that were clotted or hemolyzed, or those cases where patients were not accessible for repeat sampling, were excluded.

Patients who declined to provide their consent for participation were also excluded.

Blood Sample Collection and Analysis:

Blood samples were collected through venous puncture, employing a tourniquet to enhance venous visibility. These samples were directly introduced into EDTA-containing tubes, where they were immediately mixed with the anticoagulant. After thorough mixing on a blood shaker for a duration of 10 minutes, a complete blood count (CBC), inclusive of platelet count, was performed using the Erba-H560 cell counter. The automated methodology on the Erba 5-part analyzer cell counter hinges on the principles of electronic impedance for precise cell counting. The

analysis parameters underwent rigorous standardization through routine internal and external quality control assessments. Concurrently, fresh EDTA blood samples were employed to make peripheral blood smears, post-proper mixing on a shaker. These smears were meticulously stained using Leishman's stain. By identifying regions where red blood cells (RBCs) were juxtaposed, platelet counts were performed beneath an oil immersion lens (100x) of the Olympus cx21i microscope. The count obtained from 10 fields was then multiplied by 20,000 to yield the estimated platelet count.

Results:-

This study meticulously scrutinized blood samples from a cohort of 100 patients to estimate their platelet counts. Both automated and manual methodologies were employed for platelet count estimation..The obtained results demonstrated a remarkable degree of concordance between the two techniques.The investigation revealed a substantial correlation between the outcomes of the automated and manual platelet counting methods. The mean platelet count yielded by the automated analyzer was 1.57 lakhs, while the manual slide method produced a mean count of 2.00 lakhs.The patient cohort displayed a wide age range, spanning from a mere 1 month to a mature 82 years. The average age of the patients was calculated to be 44.6 years. These individuals were effectively categorized into three distinct groups predicated on their respective platelet counts.

Group 1: Normal Platelet Count - This group consisted of 56 patients with platelet counts ranging from 1.5 to 4.5 lakh/mm³.
Group 2: Thrombocytopenia - A cohort of 33 patients fell within this category, characterized by platelet counts below 1.5 lakh/mm³.
Group 3: Thrombocytosis - The remaining 11 individuals exhibited a platelet count surpassing 4.5 lakh/mm³, categorizing them within this group.

By delving into these distinct subgroups, the study gains an enriched perspective on platelet count variations, offering valuable insights into the diversity inherent within the patient population.

Table I:- Normal platelet count samples from manual method and automated analyzer (N=56).

	Manual Method (in Lakhs)	Automated Analyzer (in Lakhs)
1	1.8	1.33
2	2	1.62
3	2.4	2.23
4	2.6	2.51
5	3.2	2.92
6	2.2	2.11
7	3	2.89
8	2.6	2.37
9	3	2.74
10	2.8	2.48
11	3.2	2.93
12	3	2.91
13	2.8	2.61
14	2.6	2.15
15	2.8	2.33
16	2.2	1.76
17	2.4	2.02
18	2	1.87
19	3.4	3.13
20	3	2.65
21	2.6	2.41
22	2	1.88
23	2	1.74
24	2.4	2.15
25	2.5	2.18

26	3.7	3.43
27	3.4	3.19
28	2	1.86
29	4.4	4.07
30	3	2.79
31	2.8	2.4
32	3.2	2.94
33	3.2	2.96
34	2.2	1.9
35	1.8	1.7
36	1.8	1.64
37	1.9	1.66
38	1.8	1.72
39	2.8	2.57
40	1.8	1.62
41	2.6	2.45
42	2.8	2.49
43	3.6	3.23
44	3	2.79
45	2.2	1.95
46	2.6	2.32
47	3.7	3.33
48	1.8	1.62
49	2.6	2.31
50	3.4	3.16
51	3.2	2.84
52	3.4	3.16
53	3	2.77
54	2.8	2.6
55	2.6	2.44
56	2.2	1.8

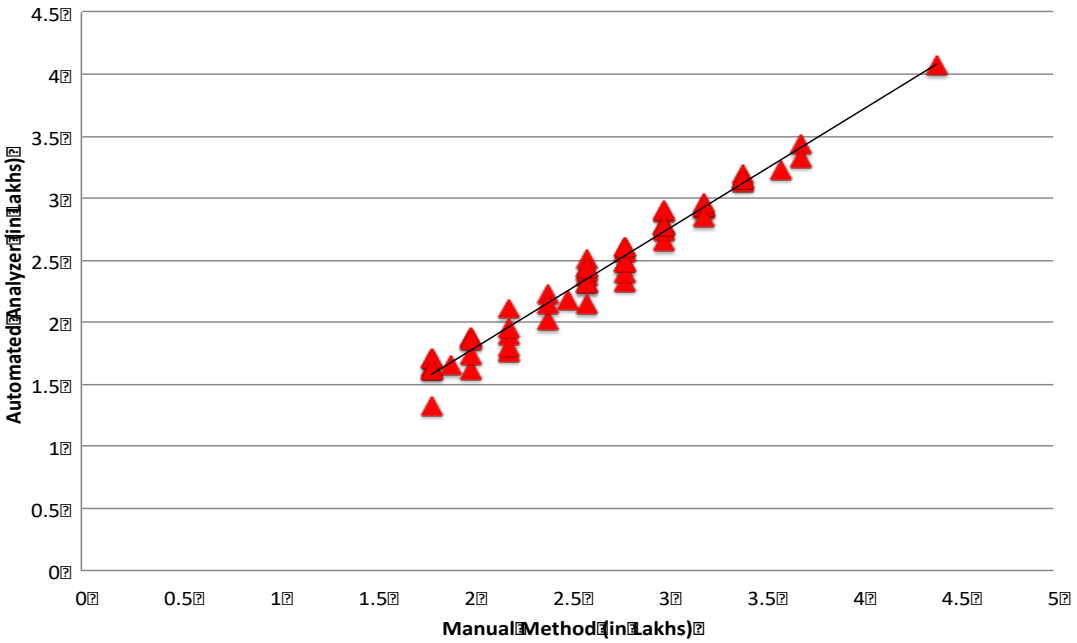

Figure 1:- Correlation between automated versus manual method pertaining to normal platelet count (N=56).

Table II:- Low platelet count samples from manual method and automated analyzer (N=33).

	Manual Method	Automated Analyzer
1	80000	33000
2	70000	36000
3	120000	60000
4	130000	65000
5	60000	33000
6	40000	16000
7	44000	30000
8	120000	56000
9	130000	68000
10	140000	54000
11	120000	70000
12	86000	50000
13	84000	49000
14	48000	28000
15	28000	17000
16	120000	37000
17	150000	78000
18	80000	60000
19	60000	34000
20	46000	28000
21	30000	10000
22	120000	44000
23	140000	63000
24	44000	17000
25	50000	20000
26	80000	44000
27	140000	73000
28	120000	78000
29	140000	86000
30	130000	70000
31	140000	90000
32	120000	77000
33	86000	40000

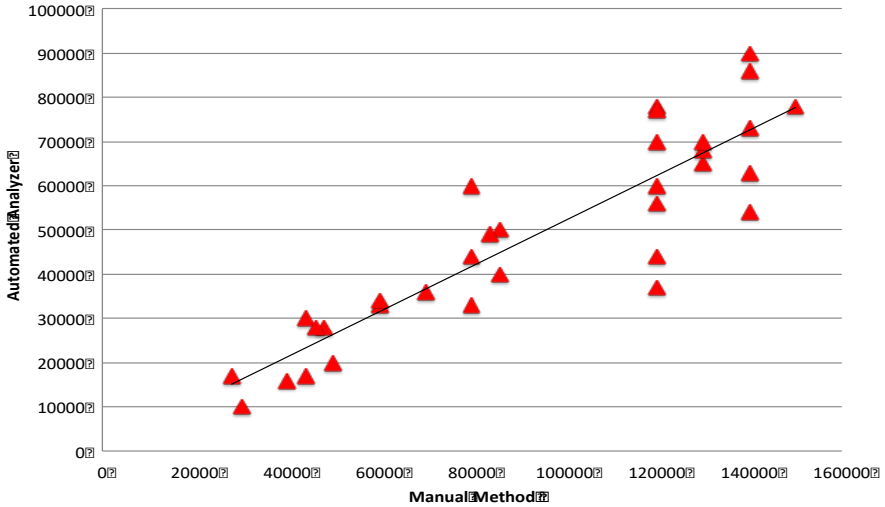

Figure 2:- Correlation between automated versus manual method pertaining to low platelet count (N=33).

Table III:- High platelet count samples from manual method and automated analyzer (N=11).

	Manual Method (in Lakhs)	Automated Analyzer (in Lakhs)
1	6.4	4.8
2	6.8	5.2
3	5.5	4.7
4	6	4.9
5	8	6.3
6	9.2	7.1
7	8.4	6.2
8	7.6	6.2
9	5.4	4.5
10	6	4.9
11	6.6	5.8

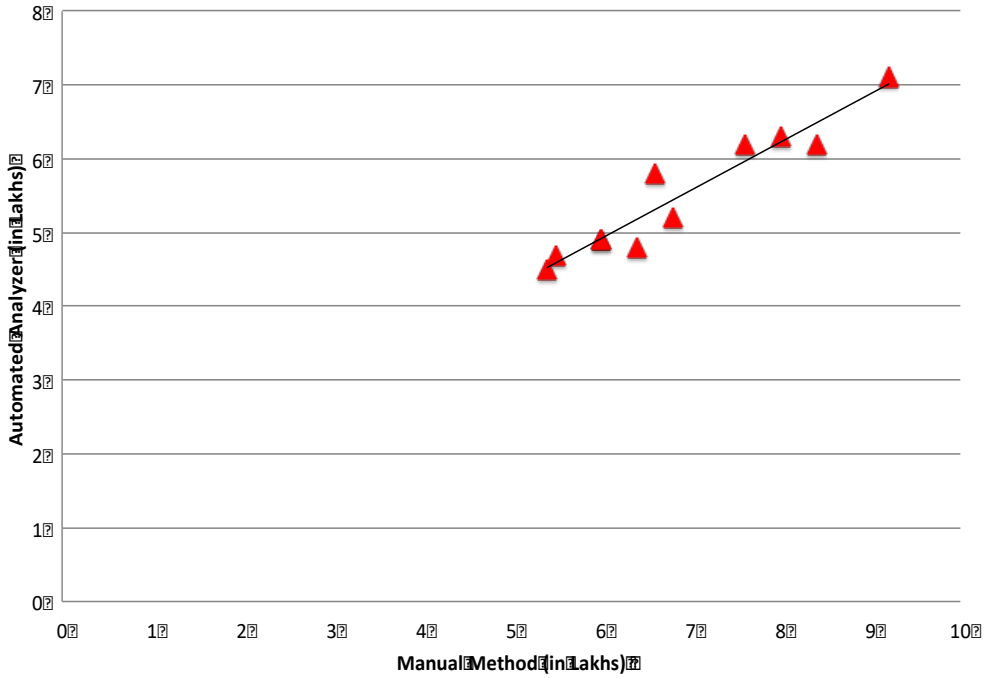

Figure 3:- Correlation between automated versus manual method pertaining to high platelet count (N=11).

Discussion:-

Platelets are small cytoplasmic protrusions with discoid morphology, having a short lifespan of merely 7-10 days, culminating within splenic macrophages[8].The pioneering contributions of Giulio Bizzozero, an esteemed Italian pathologist, are commemorated for elucidating the pivotal role of platelets within the intricate tapestry of blood clotting mechanisms[9]. Beyond mere quantification, the assessment of platelet counts is profoundly prognostic and diagnostic, unfurling its importance within the clinical landscape. Multiple methodologies coexist for platelet quantification, encompassing manual enumeration, automated cell counting, platelet enumeration through peripheral blood smears, immunoplatelet quantification, and even radioisotope labeling methodologies[10].While the gold standard once rested with manual phase contrast microscopy, its eventual abandonment was attributed to its time-consuming nature and its lack of precision in dealing with lower counts[11]. Lawrence J.B.'s exploration of the reliability of platelet counts by comparison with the manual method encapsulates the endeavor to uncover the congruence and disparities between these methodologies[12].The manual platelet count estimation proves its superiority, particularly in cases of severe thrombocytopenia or thrombocytosis, as compared to automated methods. Although automated hematology analyzers yield accurate blood counts, they may falter in enumerating platelets accurately due to factors such as particles of similar size and light scatter properties (like microcytic red cells and white blood cell fragments), as well as the presence of giant platelets and platelet clumps or aggregates. The present

study has shown that manual methods are reliable to validate automated methods under standard conditions. Leishman's stain smears, despite their utility, are limited by staining artifacts and staining inadequacies. To mitigate errors and discrepancies in haematological parameter reporting, adherence to standard guidelines necessitates processing samples within six to eight hours of collection[13,14].This study was designed to unravel the concordance between manual and automated methods in platelet count estimation. Prior research by De la Salle BJ et al. unearthed that approximately 67% of automated analyzer results exhibited overestimation, with statistically significant differences observed in 16.5% of cases[15]. Bakhubaira S in 2013 underscored a significant positive correlation between manual and automated counting methods for platelets. Their recommendation emphasized the importance of manual estimates, particularly in cases of abnormal counts[16]. Bajpai et al. concluded that the slide method of platelet estimation showed no significant difference(p value=0.69) when compared with automated cell counter values[17]. Balakrishnan et al. also found significant correlation(0.50) between manual and automated platelet counts, advocating traditional methods and platelet:RBC ratios as viable alternatives to auto-analyzers[18]. A study by Aashna et al. in 2019 suggested that automated platelet count done by analyser had an inverse relation with mean platelet volume (MPV) and platelet distribution width. They concuded that haematology analyser is crucial for quick and accurate complete blood evaluation but the blood samples that show abnormal results or low platelet count on analyser should be confirmed by manual counts on peripheral smear[19]. Jangbhadur Singh et al. in 2020 affirmed that manual platelet counting using chambers and traditional methods using peripheral blood smear remain validated and reliable techniques for platelet counting[20]. Despite the automated analyzers' presence in the hematology laboratory, the enduring importance of the manual method remains unassailable, particularly in the context of platelet count assessment across various scenarios.

Conclusion:-

To conclude, this study has shed light on the nuanced landscape of platelet count estimation, underscoring the intrinsic interplay between manual methodologies and automated analyzers. This study revealed a noteworthy positive correlation between the manual method and the automated analyzer. However, this correlative harmony manifested limitations in scenarios of both very high and very low platelet counts. Thrombocytopenic patients, in particular, unveiled significant disparities in platelet counts attributed to the presence of platelet clumps, aggregation, or irregular distribution. This underscores the importance of meticulous platelet assessment, particularly in cases that warrant precise scrutiny.Furthermore, the study reaffirmed the significance of timely platelet quantification, advocating for assessments conducted shortly after sample collection to mitigate potential inconsistencies. A cardinal conclusion drawn from this exploration is the pivotal role of a high-quality hematology analyzer in expeditious and accurate complete blood count evaluations. However before signing out the report all the samples that show abnormal platelet counts on analyser should be reassessed using a peripheral blood smear. This additional layer of verification bolsters the precision of patient care and ensures that diagnostic conclusions are anchored in accuracy.In essence, the synergy between manual and automated platelet count estimation methodologies augments our understanding and mastery of this vital diagnostic parameter, thus fostering enhanced clinical decision-making and the delivery of optimal patient care.

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