ORIGINAL ARTICLE

Comparative Analysis of Leucocyte Count (Total and Differential) in Patients with Leucocytosis using Sysmex XN550-L Series (5 Part) Automated Analyzer and Conventional Manual Technique in a Tertiary Care Hospital in Rural Haryana

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Abstract

Normal concentration of WBCs in blood varies between 4000 and 11,000 per microliter and are classified into two variants namely granulocytes and agranulocytes on the basis of presence and absence of granules. Manual methods of calculation of TLC include- Neubauer chamber and estimating WBC on leishman stained slides whereas automated methods are based on impedance or light scattering technology. The study compared the efficacy of both these methods in statistical terms using Chi Square test. 100 cases of leucocytosis analyzed over a period of 1 year at MMIMSR, Mullana, Haryana revealed leucocytosis prevalent in 4-6 decade of life with slight male predominance. The pearson coefficient for TLC came out to be p value =0.215 (not significant) but for DLC, calculations revealed *p* values for polymorphs, lymphocytes, eosinophils and monocytes to be significant (p value =0.000 each). To conclude, standard manual methods should be advocated as special practice where there is definite need of morphological and quantitative evaluation of WBCs and to validate the automated methods by which the laboratories can optimize patient care and not as a replacement.

Key Words

Neubauer, Chi Square, Analyzer, TLC, DLC

Introduction

Leucocytes or white blood cells (WBC) are the cells, which deal with the immune mechanisms of the body and are described as heterogeneous group of nucleated cells that circulate in our blood (1,2). Normal concentration of WBCs in blood varies between 4000 and 11,000 per microliter. The WBCs are comprised of a number of sub-populations with diverse biological function. On the basis of presence or absence of granules, leucocytes are classified into two variants namely granulocytes and agranulocytes (3). Evaluation of WBCs have been important in both health and diseases. Thus, method used to determine WBCs should be reliable and accurate (4). The test for the WBC count can be done by both manual and automated methods. Among the manual methods rash calculation of TLC by Neubauer chamber or estimating WBCs on leishman stained slide are there (5). Automated methods for TLC and DLC counts are based on impedance or light scattering technology (6). Various school of thoughts exist regarding the effectivity of manual and automated methods for WBC estimation. The study aims to compare TLC and DLC values for coefficient of variation between the two techniques and calculate Pearson coefficient (*p*=value) to statistically demonstrate variations, if any.

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Material and Methods

This study was undertaken in the Hematology section of Department of Pathology in M M Institute of Medical Sciences and Research, Mullana, Ambala. A random prospective study was conducted on 100 samples of adult patients with leukocytosis over a period of 1 year; 2018 - 19. Only freshly collected specimens (not more than 3hr since collection) were considered while patients >14 years of age, poorly processed slides for manual WBC assessment specimens that were less than 2ml and clotted samples were excluded out. In manual method TLC was estimated by Neubauer Chamber method and compared with automated technique Sysmex XN 550 L-series (5-part differential). EDTA was used as anticoagulant along with DIATRO. Dil diff, DIATRO. Lyse 5P, DIATRO. Diff -5p and DIATRO. Hypo cleaner as reagents for automated procedure.

The study data was analyzed using Student-t test. Chi Square test was done to compare both manual and automated technique for estimating TLC and DLC.

Result

100 samples of patients with leukocytosis were analyzed over a period of 1 year. Majority of the patients were seen in 4th – 6th decade with male to female ratio of 1.1:1. Majority of the patient's TLC both by automated and manual technique was within 11,000-21,000/ cumm range *(Table 1).* The percentage of Polymorphs ranged from 80-100% in more than half of the patients irrespective of whether done by manual or automated method *(Table 2).*

The coefficient of correlation between the manual method and automated method was calculated by using formula r2 = 0.998 coefficient of variation. The mean ±SD of TLC result by manual method was, 20526.0 ±7642.6 whereas that of automated methods was 20590.0±7714.8 Comparative study of DLC both with manual as well as automated method showed no significant difference with p value=0.215 *(Table 3).* The coefficient correlation between the manual method and automated method was calculated by using formula r2 = 0.962 coefficient of variation. The mean ±SD of DLC result by manual method was 78.0609 ± 10.8 whereas that of automated methods 79.0600 ± 12.3 Comparative study of DLC both with manual as well as automated method showed significant difference with p value=0.000 *(Table 4).* Similarly, for lymphocytes, eosinophils and monocytes the results were as 16.7500 ± 10.9, 2.3000± 1.2 and 2.4000 ± 1.18 respectively for manual method and 14.6900 ± 11.3, 2.8100 ± 1.3 and 3.0800 ± 1.43 respectively for automated method, which showed significant difference with p value=0.000 for all three lineages *(Figure 1).*

Table 1: Comparison of TLC by Manual and Automated Technique

TLC Group/mm3	Automated Method	Neubauer Chamber
4000-11000	0	0
11000-21000	66	67
21000-31000	26	25
31000-51000	7	7
Above 51000	1	1

Table 2: DLC (Polymorph) Comparison by Manual and Automated Technique

Polymorph % Range	Automated	Manual
80%-100%	54	55
60%-80%	40	41
40%-60%	5	3
Below 40%	1	1

Table 3: Mean ± SD of TLC Result by Manual and Automated Methods

Parameter	Manual	Automated	P-value	Coefficient of Variation
TLC	20526.0 ±7642.6	20590.0 ±7714.8	0.215	.998

Table 4: Mean ± SD of DLC (Polymorphs) Result by Manual and Automated Methods

Parameter	Manual	Automated	P-value	Coefficient of variation
Polymorph	78.0609 ± 10.8	79.0600 ± 12.3	0.000	.962

Figure 1: Comparison Mean ± SD of DLC (lymphocyte) by Automated and Manual Method (R=0.946, P= 0.000)

Discussion

Evaluation of leucocytes can be done through several techniques of varying complexity and sophistication. The test for the WBC count (TLC, DLC) can be done by both manual and automated methods. Diseases are characterized by changes in individual blood parameters which are more or less typical for the underlying disease. Therefore, measurement of these parameters at any time with high precision, reproducibility, and high accuracy allows a precise diagnosis (7). In this study manual count for TLC & DLC was done by Neubauer chamber and slide method respectively. For automated count in the study estimation of both TLC &DLC was done on Sysmex XN 550 L-series (5 Part differential) based on the principle of electrical impedance and light scatter. Male: Female ratio in the study of 1.1:1 was in accordance to a study conducted by Babadoko *et al.* that showed male preponderance too (8). Pearson coefficient for both the methods for estimation of TLC, the results came out as statistically not significant; p value was observed to be >0.05. In contrast to the study, studies done by Pursnani *et al.* (9) showed manual method to be inferior as compared to automated method. However, study by Karem *et al.* (10) showed comparable results with both the method that was according to this study. Momodu (11) reported that when manual method was compared to automated method fairly strong positive correlations were observed for white blood cell counts (r= 0.6828 - 0.7321, P<0.05) which was not in accordance to that reported in this study. For DLC; p value was observed to

be 0.001 which is (P < 0.05); hence statistically significant results were obtained in this study which were in contrast to reports by Lewis and Bentley (12) and previous report by Takubo and Tatsumi (13) as they documented the inability of the automated machine to identify or differentiate the leucocytes, more especially the immature cells. However, in this study we did not encounter such discrepancies owing to large proportion of our samples being within normal limits.

In our study results revealed that the manual method was preferable as compared to auto-mated for DLC determination in concordance to reports by Chung *et al.* (3) which considers automated procedures to be inferior to manual techniques in relation to reliability and identifying morphologic abnormalities and the financial concerns. However, some authors such as Babadoko *et al.* (8) and Faheem *et al.* (14) concluded that the usage of automated method has the advantage of higher accuracy and speed over manual techniques as compared to manual that are laborious and error prone. Gulati *et al.* and Ghaznavi *et al.* also documented similar findings, which were not in concordance with our study (15,16).

Hence, to conclude that although, standard manual methods give more additional information, these methods are to be warranted only to validate the automated methods by which the laboratories can optimize patient care and not to be practiced as a replacement. Thus, the manual methods are to be advocated as a special practice where there is definite need of morphological and quantitative evaluation of WBCs. In addition, manual methods are cheaper and friendly since they only involve a minimal laboratory set up and despite of the advancements that have been made regarding usage of automated methods for TLC and DLC, microscopy and morphological expertise remain the gold standard basis of diagnostic hematology (17,18).

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