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Understanding Variation in the Value of Esr in Anaemic Patients Using Different Automated Analysers And Its Comparison With Gold Standard Westergren Method

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Abstract

This study was conducted on 500 samples in the Department of Haematology, Dr Lal path labs, National Reference Laboratory, Rohini, Delhi, to analyse the variation in the value of ESR in anaemic patients using different automated analysers and its comparison with gold standard, Westergren method. Samples with Hb < 11gm% and hct < 35% were included in the study, these samples were initially analysed in automated ESR analysers- AlcoriSed as (method 1) and then in Alifax test1 analyser as (method 2) and subsequently by the manual Westergren method. Fabry's formula was used to correct the Westergren ESR.

The observed ESR was graded as normal (n=110), Intermediate (n=192), High (n=194) and very high (n=4). The mean difference between corrected ESR and Alcor iSed(Method 1) for normal, intermediate, high and very high was 22.8, 39.1,62.3 and 93.1 mm/hr respectively. Bland Altman analysis of Alcor iSed and the Westergren method show wide limits of agreement (LOA) range from -47.2 mm/hr to +56.6 mm/hr, between the two methods, particularly at higher ESR values. The comparison between Alifax test 1 and the Westergren method observed a mean difference with LOA of 22.8 (-29.7 to 62.3),39.7 (-36.4 to 50.6), 63.8 (-48.1 to 47.7) and 92.1 (-106.5 to 36.3)mm/hr respectively for normal intermediate high and very high ESR. Bland Altman analysis of the same observed a mean difference (bias) of 5.96 mm/hr with LOA ranging from -42.1 mm/hr to +54.0 mm/hr, indicating a considerable spread in differences. The mean difference (bias) between the two automated analysers of -1.24 mm/hr suggests a slight difference in estimation of ESR values by the Alcor iSed compared to the Alifax test1.

In referral laboratories with copious sample loads, the automated ESR analysers can safely replace the Westergren method for normal to intermediate ESR values in anaemic patients. The result deteriorates as ESR values increase (>100mm/hr) leading to greater variability and reduced correlation in the high and very high ESR.

In anaemic patients with high ESR (>100mm/hr), confirmation and verification by the standard Westergren method may be required.

Introduction

Erythrocyte Sedimentation Rate (ESR) was first reported in 1894 by Dr Edmund Biernacki, independently thereafter by Hirszfeld, Fåhraeus, and Sir Westergeren who popularized the parameter[1]. It evaluates the length of red blood cells falling in millimetres in a vertical column after one hour of standing and measures all three stages of sedimentation [2]. Though debated, it is one of the frequently used laboratory parameters for inflammatory conditions like giant cell arteritis, rheumatic arthritis, polymyalgia and other connective tissue disorders, infection, trauma and malignant pathologies [3]. Furthermore, ESR is considered one of the prognostic markers for disease relapse in post-chemotherapy Hodgkin Lymphoma [4]. Since its discovery, ESR was measured using the manual Westergren method, expressed as mm/1st hour, gradually being replaced by automated analysers with the advent of technology.

The Working Group by ICSH classifies the techniques of ESR estimation into three groups - Manual Westergren method, Modified Westergren method and alternate ESR method [5]. The Westergren method of ESR estimation is recommended as a gold standard method by the International Council for Standardization in Hematology (ICSH) [6,7]. With the revolution in technology, most laboratories have replaced the traditional Westergren method with automation given higher throughput, safety, negligible biohazard risk and a short processing duration. According to the ICSH global survey in 2019 by the ICSH, 28% of laboratories still use the Westergren method, while 72% of laboratories use modified Westergren or alternate methods [8]. The modified Westergren method delivers the measure of sedimentation within a brief duration of 20 to 30 minutes. On the other hand, alternate methods exploit the rouleaux formation using techniques such as photometric rheology [9] capillary photometric-kinetic technology 10 or centrifugation [11]. Results from these approaches can differ significantly from observations obtained with the Westergren method and from each other. ICSH recommends that all new technologies, instruments, or methodologies be evaluated against the Westergren reference method before being introduced into clinical use [5]. Also, it was recommended that the "systems which give the results same as the Westergren method with diluted blood at 60 minutes or normalized to 60 minutes are the only ones of clinical value" [7].

Alcor iSed is an automated analyser based on alternate Westergren- Advanced Rheology Technology that measures the "earliest and most critical phase"- Rouleau formation. This technology directly measures the aggregation of RBCs and determines the length at which the red cells will sediment in the Westergren tube. Alcor iSed draws 100µL volume and can produce a result in as little as 20 seconds with an analytical range of 2-130mm/hr [12], On the other hand, Alifax test 1 is a closed system alternate ESR analyser based on the photometric method. The blood samples are mixed slowly

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for 120 seconds and then 150 µL of blood samples transferred to the capillaries, and are measured photometrically at 950 nm wavelength. Its analytical range is 2-120mm/hr. Since ESR performed by the manual standard Westergren method is also affected by haematocrit. Fabry's formula (Westergren ESR X 15/55-HCT) can be used to correct ESR values obtained by the manual method [13].

The normal range of ESR ranges in adults from 2 to 20 mm/hour [5]. In clinical scenarios, ESR depends mainly on the haematocrit levels and plasma protein concentrate besides many other interfering factors. Many studies have been conducted to understand the role of plasma proteins as an index of inflammation. Also, a few studies have been conducted to compare two methods of ESR estimation using alternate analysers and the manual Westergren method in two different sites/institutes. In this study, we analyse the difference between the automated ESR analysers- Alcor iSed, Alifax test 1 with the manualWestergren method, corrected by Fabry's formula for the entire range of ESR amongst anaemic patients in a single stand-alone referral laboratory in Delhi.

Materials and Method

Sample collection

This study was conducted at Dr Lal path labs, National Reference Laboratory, Rohini Delhi. Sample size was calculated under 95% confidence limit using the formula, n=4s2/d2 where s=mean and d= margin of error. Based on earlier result of similar study14 with mean= 21.3, margin of error as 2, a sample size of 454 was obtained. However, to get an optimal result, a sample of 500 was taken for the study. 500 random samples with haemoglobin less than 11gm% and HCT<35% and an adequate volume sent to the Department of Haematology for routine tests were collected after the routine tests had been performed. Samples were initially analysed in automated ESR analysers- AlcoriSed as method 1 and then in Alifax test1 analysers as method 2 and subsequently by the manual Westergren method using Westergren pipette. For manual estimation of ESR, the EDTA samples were diluted as per the guidelines by the ICSH in the ratio of 4:1 of blood and 3.8 % trisodium citrate dihydrate, mixed and then filled into the Westergren tubes. Samples were placed vertically in the tube holder at room temperature in an area free from vibration. Erythrocyte sedimentation was visually read and recorded as an absolute number after 1 hour of being laid. The manual Westergren values were corrected using Fabry's formula for low hematocrit (Westergren ESR X 15/55-HCT). The results were further categorized into four groups in this study as value up to 20 mm/hour as normal and increased ESR (20 mm/hour and above) were further sub categorised into intermediate (ESR: 20 to less than 50mm/hour); high (ESR: 50 to less than 100 mm/hr) and very high (ESR greater than or equal 100 mm/hr). Any degenerated samples and inadequate samples were excluded from the study.

Statistical Analysis

Data were described in terms of range, mean ± standard deviation (SD), frequencies (number of cases), and relative frequencies (percentages) as appropriate. All the entries were entered in a Microsoft Excel sheet. Simultaneous comparison of Alcor iSed with the corrected manual ESR and Alifax test1 was done using Bland-Altman plots. 95% limits of agreement were calculated as d ± 1.96 SD, where d = mean difference between the two measurements and SD = standard deviation of differences. Pearson correlation was used to find the correlation among various parameters. A probability value (*p-value*) less than 0.05 was considered statistically significant. SPSS software version 21.0 was used for statiscal analysis.

Results

This study conducted on 500 samples with anaemia with Hb < 11% and that of hct < 35% reveals a mean (SD) age of the study participants of 53.3 (±18.1) with a minimum of one year and a maximum of 91 years. A higher proportion of female participants (67.6%), compared to male participants (32.4%) was observed.

The haematological parameters exhibited considerable variation across the sample. The mean haemoglobin (Hb) level is 9.4g/dL with a standard deviation (SD) of 1.3, ranging from 3.5 to 10.9 g/dL. Mean haematocrit (Hct) levels were 29.3% (SD = 4.2), spanning from 12.9% to 35%. The mean corpuscular volume (MCV) has a mean of 81.6 fL (SD = 12.6),ranging between 48.7 and 134.9 fl, highlighting diverse erythrocyte sizes within the population.

In the normal ESR group, comparison of the two automated analysers (Alcor iSed and Alifax test 1) had the same mean difference of 22.9mm/hr with the corrected ESR under 95% confidence limit (Table 1, 2). Amidst the two automated analysers. a statistically significant correlation with a mean difference of 31.02 mm/hr was observed. In the intermediate ESR group, a mean difference of 31.9 mm/hr between Alcor iSed and the corrected ESR; 39.7 mm/hr between Alifax test1 and the corrected ESR was observed. A considerable variability suggested by the wide limit of agreement (LOA) in both the automated analysers- Alcor iSed ranging from -40.4 to 52.1 mm/hr along with a correlation coefficient of 0.18 (p = 0.01), and alifax test1 from -36.4 to 50.6 mm/hr and a lower correlation of 0.24 (p = 0.001) implied a weaker agreement was agreement between automated analysers and the manual method. However, amongst the

Table 1: Comparison of Corrected Manual ESR and Alcor (Method 1) ESR: Mean Difference, 95% Limits of Agreement, and Correlation Coefficient

ESR group	Mean difference between corrected ESRand Alcor (Method 1) value (mm/hr)	95% limit of agreement	Correlation coefficient	p-value
Normal (n=110)	22.8 (26.1)	-32.8 to 69.6	0.38	**0.01**
Intermediate (n=192)	39.1 (23.5)	-40.4 to 52.1	0.18	**0.01**
High (n=194)	62.3 (25.5)	-53.4 to 46.8	1	0.07
Very high (n=4)	93.1 (34.7)	-101.1 to 34.9	1	0.2

Table 2: Comparison of Corrected Manual ESR and Alifax (Method 2) ESR: Mean Difference, 95% Limits of Agreement, and Correlation Coefficient

ESR group	Mean difference between corrected ESRand Alifax (Method 2) value (mm/hr)	95% limit of agreement	Correlation coefficient	p-value
Normal (n=110)	22.8 (23.5)	-29.7 to 62.3	0.42	**<0.001**
Intermediate (n=192)	39.7 (22.2)	-36.4 to 50.6	0.24	**0.001**
High (n=194)	63.8 (24.4)	-48.1 to 47.7	1	0.08
Very high (n=4)	92.1 (36.4)	-106.5 to 36.3	1	0.39

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two analysers, the mean difference was 42.6 mm/hr with limits of -1.1 to 86.3, a correlation coefficient of 0.86, and a significant p-value of 0.001.

The comparison between Alcor iSed and the manual method in the high ESR group(n=194) exhibited a mean difference of 62.3 mm/hr, with a wide LOA (-53.4 to 46.8 mm/hr) and a correlation coefficient of 1, statistically not significant (p = 0.07). Between Alifax test1 and the manual Westergren method, the high ESR group showed an even greater mean difference of 63.8 mm/hr, but the LOA (-48.1 to 47.7 mm/hr) suggested a wide variation, and the correlation drops to 1, statistically insignificant, p-value (0.08). Between the two automated analysers, a statistically significant p=0.001, with a mean difference was 62.2 mm/hr, and 95% limit of agreement from 15.6 to 108.8, a correlation coefficient of 0.83 was observed (Table 3).

Whereas the very high ESR group (n=4) demonstrated the largest mean difference of 93.1 mm/hr, with an LOA ranging from -101.1 to 34.9 mm/hr, a correlation coefficient of 1, and statistically insignificant (p = 0.2) between Alcor iSed and the westergren method. Similarly, a mean difference of 92.1 mm/hr, with an extremely wide LOA (-106.5 to 36.3 mm/hr), between Alifax test1 and the manual westergren method indicating poor agreement, a correlation coefficient of 1 with a non-significant p-value (0.39). Between the two automated analysers,the mean difference was 75.5 mm/hr, with limits ranging from 18.5 to 132.4, a correlation coefficient of 0.94, and a p-value of 0.05. Four entries went beyond the analytical range of Alcor iSed as compared to five entries in Alifax test1. These findings suggest that the agreement between automated analysers and corrected ESR is relatively better at lower ESR levels, it deteriorates as ESR values increase, leading to greater variability and reduced correlation in the high and very high ESR ranges.

The Bland-Altman plot assessment between Alcor iSed and the westergren method observed a mean difference (bias) of 4.71 mm/hr suggesting an overestimation by this automated method slightly than the manualmethod as compared to the mean difference (bias) of 5.96 mm/hr between the Alcor iSed and the manual Westergren method. A poor correlation exists when ESR increases beyond 100mm/hr in anaemia between the automated analysers and the manual method. A few outliers fall beyond the LOA, further highlighting inconsistencies. (Figure 1).

The Bland-Altman plot of Alifax test 1 and the manual Westergen method observed a LOA ranging from -42.1 mm/hr to +54.0 mm/hr, with a considerable spread in differences, suggesting reduced agreement between the methods as ESR levels rise. Some outliers exceed the LOA, emphasizing further inconsistencies. Although the overall bias is small, the broad LOA indicates limited interchangeability between the two methods, particularly in higher ESR ranges where variability is more pronounced (Figure 2). The mean difference (bias) between the two automated analysers of -1.24 mm/hr suggests a minimal difference in estimation of ESR values by the Alcori Sed compared to the Alifax test1. The comparison of the two automated analysers by Bland Altman is plotted in (Figure 3).

Table 3: Comparison of AlcoriSed (Method 1) and Alifax test1 (Method 2) ESR: Mean Difference, 95% Limits of Agreement, and Correlation Coefficient

ESR group	Mean difference between Alcor iSed (Method 1) and Alifax test1 (Method 2) value (mm/hr)	95% limit of agreement	Correlation coefficient	p-value
Normal (n=110)	31.02 (26.1)	-20.2 to 82.2	0.94	**0.001**
Intermediate (n=192)	42.6 (22.3)	-1.1 to 86.3	0.86	**0.001**
High (n=194)	62.2 (23.7)	15.6 to 108.8	0.83	**0.001**
Very high (n=4)	75.5 (29.1)	18.5 to 132.4	0.94	0.05

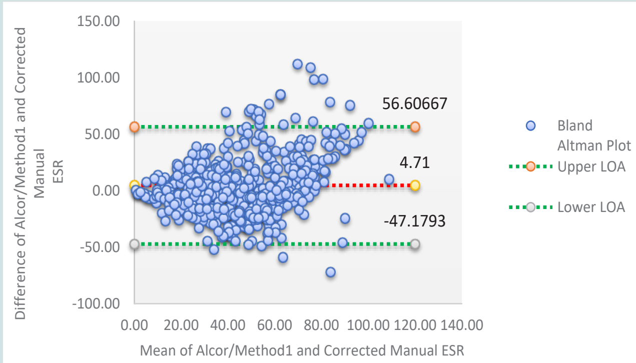

Figure 1: Bland-Altman Analysis of Alcor (Method 1) ESR vs. Corrected ESR: Mean Difference = 4.71, 95% Limits of Agreement (-47.1 to 56.6).

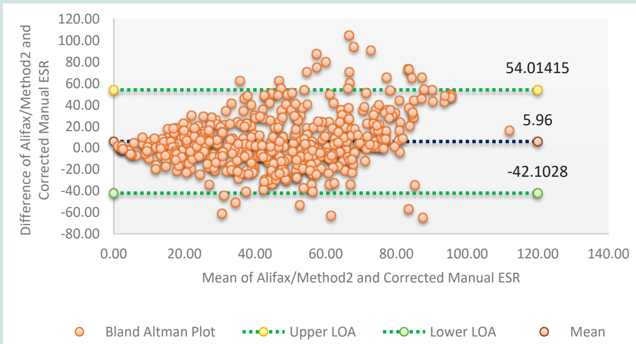

Figure 2: Bland-Altman Analysis of Alifax (Method 2) ESR vs. Corrected ESR: Mean Difference = 5.96, 95% Limits of Agreement (-42.1 to 54.01).

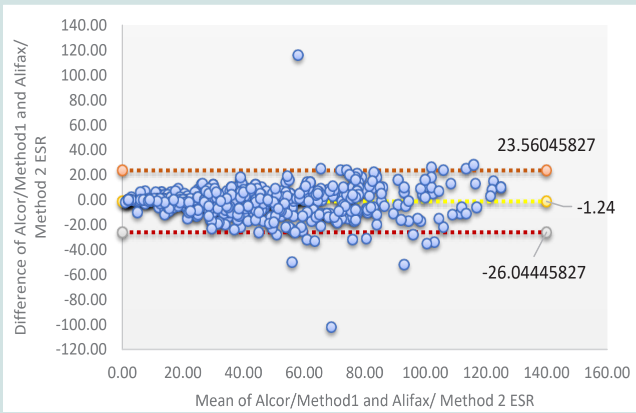

Figure 3: Bland-Altman Analysis of Alcor (Method 1) vs Alifax (Method 2) ESR: Mean Difference = -1.24, 95% Limits of Agreement (-26.04 to 23.56).

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Discussion

The distribution of age groups among the study participants revealed that the majority fall within the 61-70 years category (19.2%) a significant representation of older and middle-aged adults. Younger individuals and the paediatric population are less represented, with only 7.2% in the 21-30 years category and 3.4% under 20 years. This trend may be possibly due to the use of CRP instead of ESR as a marker of inflammation in the paediatric population [15].

The female participants (67.6%), accounted for two third of the study population, indicating a significant gender disparity, with females making up more than two-thirds of the total sample, similar to a study by Cennamo et al [16].This is probably due to the inclusion criteria of samples of the study as anaemia is known to be more prevalent among females. As per the latest release by WHO on Feb 2025 and the Global health metrics, "An estimated 106 million women and 103 million children affected by anaemia in Africa and 244 million women and 83 million children affected in South-East Asia. With women contributing more than male [17].

This study also observed the difference between corrected ESR and ESR analysed by automated analysers increased as the ESR value increases. A greater variability between the corrected Westergren ESR and the two automatedanalysers value was observed as the value of ESR increased. This is in concordance with a study by Narang et al [10]. Sonmez et al [14]. Hence, Fabry's formula was applied to correct the overestimation. Fabry's formula corrected the ESR value in the low and intermediate range, however, a persistent significant difference was observed for high and very high ESR ranges.

For all the range of ESR, the agreement between the two automated analysers- Alcor iSed, Alifax test1 in comparison with the gold standard westergren method was relatively better at lower ESR levels, deteriorated as ESR values increased, leading to greater variability and reduced correlation in the high and very high ESR ranges. This weakens agreement of the two automated analysers with the manual Westergren highlighted a greater variability and poorer correlation, making the two methods less interchangeable in high ESR group. These findings are concordant with Sonmez et al [14] that found a poor correlation between the automated analyser and manual methods at high ESR values ($p > 0.10$). Dhruva et al [18] also observed that samples with high ESR values vary considerably around the mean difference compared with samples that had normal ESR readings.

Subramaniam et al [19] observed a wide LOA (between −57.3 to 30.5 for high values) and a narrow LOA (−18.9 to 3.5 for normal ESR) while comparing automated instrument MONITOR 100 from Electa Laboratory Italy and the manual method. Likewise, Alfadhli et al [20] showed low agreement between the automated and Westergren methods at the higher ESR values as compared with normal ranges. They also reported that for samples with ESR readings > 25 mm/hr ($n = 81$), the mean of difference (−21.4) and the 95% limits of agreement (−45.2 and 2.26) were markedly different from the corresponding values (−3.9, −13.5 and 5.7, respectively) for samples with ESR values < 25 mm/h ($n = 69$).

Anaemia and low haematocrit do impact the ESR values of different automated analysers for all the ranges of ESR. Bland and Altman analysis of the two automated analysers with the manual Westergren method showed a poor correlation of the ESR in anaemic patients at high ESR (>100mm/hr). There is a better agreement in the low and intermediate ESR ranges, with differences clustering around the mean, the scatter increases significantly at higher ESR values, suggesting poor correlation in this range. A few outliers fall beyond the LOA, further highlighting inconsistencies.

Conclusion

In large referral laboratories with copious sample loads, the automated ESR analysers can safely replace the Westergren method for normal to intermediate ESR values in patients with low hematocrit.

However, in anaemic patients with higher ESR(>100mm/hr), the result may require confirmation and verification by the standard Westergren method with correction using Fabry's formula**.** A simple step of double checking automated ESR readings with the manual westergren method, corrected by Fabry's formula would be worthwhile, and ensure a superior, trustworthy quality report.

Limitation of the study

The samples were procured after the routine tests had been performed. Most cases of severe anaemia were excluded from the study due to inadequacy of sample volume and to avoid prolonged storage of sample that may impact the result of the study.

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