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ERYTHROCYTE SEDIMENTATION RATE: COMPARATIVE ANALYSIS OF MANUAL AND AUTOMATED TECHNIQUES IN A TERTIARY CARE HOSPITAL.

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ABSTRACT

Objective: Erythrocyte Sedimentation Rate (ESR) test is a frequently ordered non specific marker of infection and inflammation. The Westergren method is the gold standard method but it has many disadvantages including posing biohazard risk to lab personnel. The automated methods overcome these limitations and provide rapid results with improved standardisation. This study aimed to compare the ESR values of an alternate method of ESR measurement which works on principle of capillary photometry and the Westergren method. **Material & Methods:** ESR values of 516 samples in the age range of 12 to 98 years and gender ratio of 1:1.2 (Male : Female) were obtained by Westergren method and Alifax Test 1 analyser and compared for ESR ranges, hematocrit (HCT), hemoglobin (Hb) and mean corpuscular volume (MCV). **Statistical Analysis:** Passing Bablok, Bland Altman and Spearman's correlation coefficient (r) were used as statistical tools. A p-value of < 0.05 was accepted as statistically significant and all the data was analysed using the SPSS 25 version. **Results:** The ESR values obtained by the automated method showed higher mean values as compared to the Westergren method. A strong positive correlation between the two methods ($r = 0.852$, p-value <0.001) was observed overall. The analysis amongst various subcategories also yielded positive correlation. However in the very high range of ESR (>70 mm/h), a very poor correlation coefficient of 0.161 (p-value of 0.239) was observed. **Conclusion:** Test 1 analyser and the gold standard Westergren method demonstrated a good correlation for ESR estimation. The automated method is faster, safer and can efficiently help optimize time and lab resources, especially in high-volume laboratories. It also performs well with varying ranges of Hb, HCT and MCV.

KEYWORDS

ESR, Westergren, Alifax Test 1, ESR Automated Analyzer, Erythrocyte Sedimentation Rate.

INTRODUCTION

Erythrocyte sedimentation rate (ESR) estimates the rate at which erythrocytes settle at the bottom of a vertical column in a one hour period. It is a physiochemical phenomenon involving suspension of erythrocytes based on red cell surface charge as well as composition of various plasma proteins [1–5]. Even though ESR has low specificity and cannot help identifying the underlying pathological cause of a disease, it still remains as one of the routinely requested tests in a clinical pathology laboratory [6–8]. It is useful in various infectious, acute & chronic inflammatory, autoimmune and malignant conditions. ESR aids in diagnosis, is a part of prognostic criteria and helps in monitoring disease activity, therapeutic response and disease relapse [9–13].

ESR was first described by Dr. Edmund Biernacki in 1894 and later on Dr. A. V. A. Westergren developed a standardized Westergren method for measuring ESR in 1921, which was first adopted as the gold standard method of ESR measurement by International Council for Standardization in Hematology (ICSH) in 1973 and then also by Clinical Laboratory Standards Institute (CLSI) [1,14–17]. The Westergren method is sensitive and reliable, however it is time and labor intensive, which in turn increases its turn around time (TAT) [4,12,13]. It is also affected by many factors like size and shape of erythrocytes, hematocrit (HCT), precise ratio of blood and anticoagulant as well as many environmental and procedural factors like temperature, tilting and vibration [7,11,14,15].

Automation of ESR measurement is being introduced in laboratories at a rapid pace since the last two decades. According to an ICSH survey, 72% labs worldwide have now replaced the Westergren method for a modified or alternate method of ESR measurement [14]. The newer automated methods provide various advantages over the Westergren method like faster processing time, reduce exposure risk to biological hazardous material, minimize human error, some instruments use EDTA vacutainers and thereby exclude the requirement of a separate ESR tube, increase the number of tests performed per hour especially in high workload labs [6,7,18,19]. The integration of these instruments to Hospital Information System (HIS) can also reduce the errors in transcription and can also quickly deliver the test results to clinicians [12,14].

ICSH and CLSI have defined standardized method for Westergren and have also published accepted modifications to the traditional Westergren method as well as provided recommendations for modified and alternate automation methods of ESR measurements. ICSH recommends that prior to introduction of newer methodologies of ESR measurement into a lab, they have to be validated against the Westergren method. The data obtained should have comparable results with good precision and accuracy [14]. Numerous studies have been conducted to compare Westergren method with the newer automated methods, which have yielded variable results, maximum being 142% mean difference between the two methodologies in an ICSH survey [14,20]. Various studies have also shown comparable results and advocate the use of automated methods, while some studies have observed significant differences, especially in a group of conditions like anemia, low HCT, very high ESR values, etc [3,8,9,11,15,19].

The advantages of newer automated methods for ESR estimation outweighs their limitations and hence more studies should be undertaken to improve their accuracy and precision.

The aim of this study is to compare the ESR values of an alternate method of ESR measurement which works on principle of capillary photometry and the gold standard Westergren method. The results were also compared for different ranges of hematocrit (HCT), hemoglobin (Hb) and mean corpuscular volume (MCV).

MATERIALS & METHODS

Sample Collection

The study was conducted over a period of 3 months in the Department of Clinical Pathology at a tertiary care hospital in New Delhi, India after due approval from the Institutional Ethics Committee. Samples submitted for CBC and ESR with a minimum volume of 3 ml in K2-EDTA vacutainers were randomly selected from both admitted and outpatient department patients. Following ICSH guidelines, all the samples were tested within 4 hours of collection [14]. The samples were first submitted for CBC and ESR estimation by the automated method and then analysed by the Westergren method with the leftover sample. Inadequate quantity, clotted, hemolysed or lipemic samples and samples with patient age <12 years were excluded from the study [12]. A total of 550 samples were collected, but 34 were rejected following the exclusion criteria.

Automated ESR Analyzer

Test 1 (Alifax s.r.l., Polverara, PD, Italy) is an alternate Westergren method for automated ESR measurement which works on the principle of quantitative capillary photometry [1,2,10,21]. Blood sample (175 µL) from K2-EDTA vacutainer is aspirated to a capillary tube kept at 37°C via a fixed needle. The instrument measures ESR using photometry in 20 seconds and then provides the result in Westergren data (mm/h) in a range of 2 to 120 mm/h [2,17]. All levels of quality controls provided by the manufacturer were processed regularly.

Westergren Method

The gold standard Westergren manual method of ESR measurement was performed adhering to the guidelines and recommendations provided by ICSH. Samples received in K2-EDTA vacutainers were diluted in a ratio of 1:4 (anticoagulant : blood) with liquid 3.8% trisodium citrate dihydrate anticoagulant. The sample was then gently mixed by inverting the tubes 20 times and aspirated into standard Westergren glass pipette with 2.55 mm diameter and graduated up to 200 mm. The pipette was placed in a vertical stand at an ambient temperature of 20-25°C and away from vibrations and direct sunlight [14]. The upper meniscus of the red cell column in the pipette was noted as result by the same lab technician to minimize the interobserver bias. Fabry's formula (corrected ESR = measured ESR x 15/[55-HCT]) was used to correct the results with a HCT <35% [2,3].

Statistical Analysis

ICSH recommends using Bland-Altman, Passing-Bablok regression and coefficient of correlation as statistical tools to compare the Westergren and automated methods of ESR measurement [14].

Bland-Altman analysis assesses the mean difference and constructs limits of agreement and essentially quantifies the agreement between two quantitative analytical methods [12,22]. Passing-Bablok linear regression estimates the agreement and systematic bias between two methodologies. The scatter diagram is plotted with a regression line that allows visual observation of data with their 95% confidence interval (95% CI) [12,23]. Spearman rank correlation coefficient (r) was used to measure the strength of association between the two methods [2,24].

A p-value of < 0.05 was accepted as statistically significant and all the data was analysed using the SPSS 25 version.

RESULTS

A total of 516 samples were analysed by both Test 1 (automated method) and the gold standard Westergren method for correlation of ESR values. This comparison study was done by distributing the data as follows:

- **Age:** Patients were segregated into two age groups of 12 to 25 and ≥26 years [13]. The mean age of patients in this study was 38.8 years. The first age group (12 - 25 years) consisted of 110 (21.3%) patients with a mean ESR ± standard deviation (SD) of 28.98 ± 27.69 mm/h by automated analyzer and 19.78 ± 22.55 mm/h by Westergren method. The second age group (≥26 years) had 406 (78.7%) patients with a mean ESR ± SD of 32.73 ± 27.5 mm/h by automated analyzer and 24.51 ± 23.42 mm/h by Westergren method. The first group showed Spearman's rho correlation (r) of 0.796 (p-value <0.001) and the second group showed r = 0.863 (p-value <0.001), suggesting a comparable correlation amongst both groups by both the methods (table 1, fig.1).

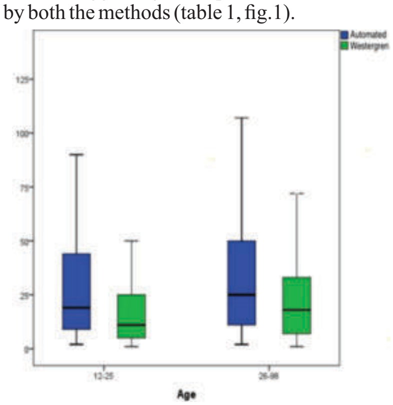

Fig.1: Box and Whisker plot for ESR measurement according to age by automated Test 1 analyzer (Median - 19 mm/h, Quartile 1 (Q1) - 9 mm/h, Q3 - 44 mm/h for age group of 12 - 25 years and median - 25 mm/h, Q1 - 11mm/h, Q3 - 50 mm/h for age group of ≥ 26 years) and Westergren method (median - 11 mm/h, Q1 - 5 mm/h, Q3 - 25 mm/h for age group of 12 - 25 years and median - 18 mm/h , Q1 - 7 mm/h, Q3 - 33 mm/h for age group of ≥ 26 years)

- **Gender:** this study included 239 male and 277 females with a gender ratio of 1 : 1.2. Mean ESR ± SD in the group with male participants was 34.54 ± 30.33 mm/h by Test 1 analyzer and 26.12 ± 25.58 mm/h by Westergren method with r = 0.87 (p-value <0.001). Mean ESR ± SD in the group with female participants was 29.68 ± 24.76 mm/h by Test 1 analyzer and 21.25 ± 20.91 mm/h by Westergren method with r = 0.821 (p-value <0.001). Both groups showed good comparability (table 1, fig.2).

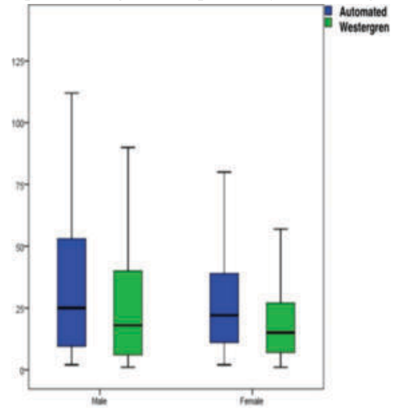

Fig.2: Box and Whisker plot for ESR measurement according to gender by automated Test 1 analyzer (Median - 25 mm/h, Q1 - 9 mm/h, Q3 - 53 mm/h for males and median - 22 mm/h , Q1 - 11 mm/h, Q3 - 39 mm/h for females) and Westergren method (median - 18 mm/h, Q1 - 6 mm/h, Q3 - 40 mm/h for males and median - 15 mm/h , Q1 - 7 mm/h, Q3 - 27 mm/h for females).

Table 1: Correlation Between Automated (Test1 analyzer) and Westergren Method Based on Age, Gender, ESR Range and MCV.

Variable	Category	N	Method of ESR measurement (mm/h)		r	p-value
			Automated (Mean ± SD)	Westergren (Mean ± SD)		
Age (years)	12 - 25	110	28.98 ± 27.69	19.78 ± 22.55	0.796	0.0001
	≥ 26	406	32.73 ± 27.50	24.51 ± 23.42	0.863	0.0001
Gender	Males	239	34.54 ± 30.33	26.12 ± 25.58	0.870	0.0001
	Females	277	29.68 ± 24.76	21.25 ± 20.91	0.821	0.0001
ESR range (mm/h)	1 - 126	516	31.93 ± 27.56	23.51 ± 23.29	0.852	0.0001
	0 - 20	232	9.56 ± 5.62	8.32 ± 7.08	0.650	0.0001
	21 - 70	229	40.43 ± 14.46	29.52 ± 19.23	0.628	0.0001
	>70	55	90.91 ± 14.99	62.49 ± 26.58	0.161	0.239
MCV (fL)	<80	84	43.11 ± 32.50	24.55 ± 23.74	0.753	0.0001
	80 - 100	395	29.97 ± 26.17	23.80 ± 23.66	0.885	0.0001
	>100	37	27.46 ± 24.09	17.97 ± 17.36	0.722	0.0001

(N-Total number of participants, SD-Standard Deviation, r-Spearman's rho correlation coefficient, MCV-Mean Corpuscular Volume)

- **ESR Values:** ESR values were divided into normal (0-20 mm/h), high (21-70 mm/h) and very high (>70 mm/h) according to the manufacturer's manual. The entire analytical range as well as all the subgroups were compared with each other for the two methodologies. Mean ESR ± SD values obtained from the entire data was 31.93 ± 27.56 by Test 1 and 23.51 ± 23.29 by Westergren's method with an r value of 0.852 (p-value <0.001) (fig.3 & 4). In the first and second subcategories, the mean ESR ± SD by Test 1 analyzers were 9.56 ± 5.62 mm/h and 40.43 ± 14.46 mm/h, while 8.32 ± 7.08 mm/h and 29.52 ± 19.23 mm/h by Westergren method, respectively. Spearman's rho correlation was relatively comparable within these two groups, as r = 0.650 in the first group and r = 0.628 in the second group. However, the data demonstrated poor comparability with very high ESR values of >70 mm/h as r = 0.161 and p-value was not statistically significant (p-value = 0.239) (Table 1).

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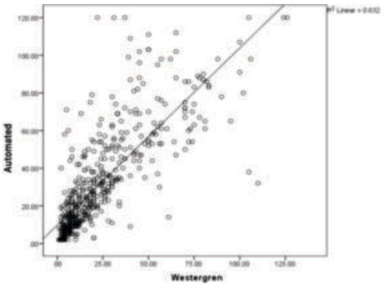

Fig.3: Passing Bablok regression analysis plot for comparison of automated (Test 1) analyzer and Westergren method for ESR measurement [regression equation y= 9.818 + 0.941x and 95% C.I. = 0.878 to 1.003).

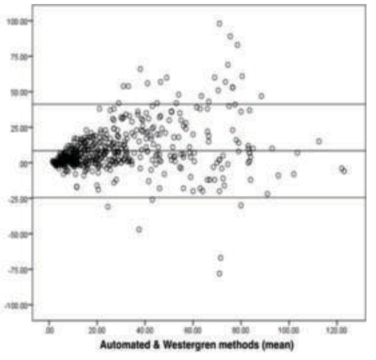

Fig.4: Bland-Altman analysis plot for comparison of automated (Test 1) analyzer and Westergren method for ESR measurement.

- **Hematocrit (HCT):** The data was divided into two groups to compare the difference between ESR values according to HCT level [4]. The first group had HCT < 35% and the ESR values of this group obtained by Westergren method were corrected using Fabry's formula. The second group had HCT ≥ 35%. The first group showed 46.12 ± 27.94 mm/h mean ESR ± SD by Test 1 while 52.58 ± 40.56 mm/h by the Westergren method before correction and 31.48 ± 23.60 mm/h after correction. Spearman's rho correlation in this group was 0.758 (p-value <0.001) before correction and 0.779 (p-value <0.001) after correction. Mean ESR ± SD in the second group was 26.06 ± 25.19 mm/h by Test 1 and 20.21 ± 22.38 mm/h by the Westergren method with r = 0.845 (p-value <0.001). The data suggests better correlation for the samples with HCT ≥35% and slightly better correlation after correction by Fabry's formula in first group (table 2, fig.5).

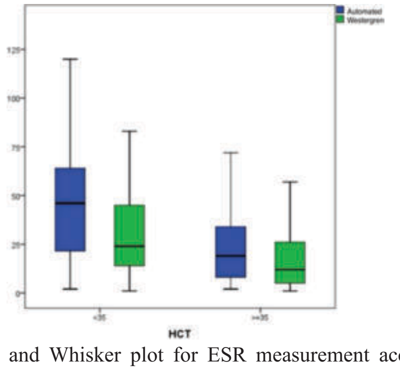

Fig.5: Box and Whisker plot for ESR measurement according to hematocrit levels by automated Test 1 analyzer (Median - 46 mm/h, Q1 - 21 mm/h, Q3 - 64 mm/h for the group with HCT <35% and median - 19 mm/h, Q1 - 8 mm/h, Q3 - 34 mm/h for the group with HCT ≥ 35%) and Westergren method (median - 24 mm/h, Q1 - 14 mm/h, Q3 - 45 mm/h for the group with HCT <35% and median - 12 mm/h , Q1 - 5 mm/h, Q3 - 26 mm/h for the group with HCT ≥ 35%).

Table 2: Correlation Between Automated (Test1 Analyzer) and Westergren Method Based on Hematocrit.

Hemato-crit (%)	Category	N	Method of ESR measurement (mm/h)		r	p-value
			Automated (Mean ± SD)	Westergren (Mean ± SD)		
< 35	Before correction	151	46.12 ± 27.94	52.58 ± 40.56	0.758	0.0001
	After correction		46.12 ± 27.94	31.48 ± 23.60	0.779	0.0001
≥ 35		365	26.06 ± 25.19	20.21 ± 22.38	0.845	0.0001

(N-Total number of participants, SD-Standard Deviation, r-Spearmen's rho correlation coefficient)

- **Hemoglobin (Hb):** The data was divided into two groups by age and sex and both groups were further subdivided into four categories of no-anemia, mild, moderate and severe anemia according to WHO classification of anemia [25]. The findings are depicted in table 3. The results showed good comparability across all groups with significant p-value.

Table 3: Correlation between automated (Test1 analyzer) and Westergren method based on hemoglobin range for different genders and age groups.

Cate-gory	Hemoglobin (gm/dL)	N (516)	Method of ESR measurement (mm/h)		r	p-value
			Automated (Mean ± SD)	Westergren (Mean ± SD)		
12-14 year old Males and >12 years Fe-males	No Anemia (≥ 12.0)	97	18.74 ± 15.98	14.53 ± 17.75	0.753	0.0001
	Mild Anemia (11.0 - 11.9)	84	27.69 ± 24.53	21.56 ± 22.41	0.772	0.0001
	Moderate Anemia (8.0 - 10.9)	89	41.71 ± 27.18	26.79 ± 21.09	0.802	0.0001
	Severe Anemia (<8.0)	12	43.08 ± 26.73	32.0 ± 23.12	0.716	0.0001
>14 years Males	No Anemia (≥ 13.0)	113	19.76 ± 22.33	13.45 ± 15.61	0.829	0.0001
	Mild Anemia (11.0 - 12.9)	58	48.17 ± 31.68	40.69 ± 29.40	0.794	0.0001
	Moderate Anemia (8.0 - 10.9)	46	49.44 ± 28.52	37.46 ± 25.19	0.832	0.0001
	Severe Anemia (<8.0)	17	46.8 ± 31.72	30.87 ± 23.0	0.625	0.0001

(N-Total number of participants, SD-Standard Deviation, r-Spearmen's rho correlation coefficient)

- **Mean Corpuscular Volume (MCV):** Three groups with MCV <80 fL, 80-100 fL and >100 fL were compared for ESR estimation by the two methods. The group with MCV <80 fL exhibited mean ESR ± SD of 43.11 ± 32.50 mm/h by Test 1 and 24.55 ± 23.74 mm/h by Westergren's method with r = 0.753 (p-value <0.001). The second group had a mean ESR ± SD of 29.97 ± 26.17 mm/h by Test 1 and 23.80 ± 23.66 mm/h by Westergren's method with r = 0.885 (p-value <0.001). The third group with MCV >100 fL showed mean ESR ± SD of 27.46 ± 24.09 mm/h by Test 1 and 17.97 ± 17.36 mm/h by Westergren's method with r = 0.722 (p-value <0.001). The p-value of all the groups is <0.001, however the best correlation was observed in the group with MCV = 80 to 100 fL (table 1, fig.6).

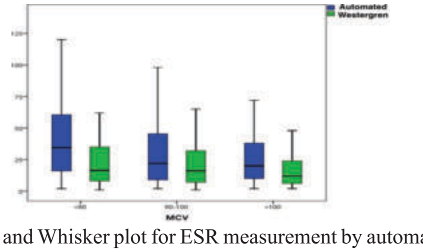

Fig.6: Box and Whisker plot for ESR measurement by automated Test

1 analyzer (Median - 34.5 mm/h, Q1 - 16 mm/h, Q3 - 60.5 mm/h for MCV <80 fL; median - 22 mm/h, Q1 - 9 mm/h, Q3 - 46 mm/h for MCV= 80 - 100 fL; and median - 20 mm/h, Q1 - 10 mm/h, Q3 - 38 mm/h for MCV >100 fL) and Westergren method (Median - 16.5 mm/h, Q1 - 8 mm/h, Q3 - 35 mm/h for MCV <80 fL; median - 16 mm/h, Q1 - 7 mm/h, Q3 - 32 mm/h for MCV= 80 - 100 fL; and median - 12 mm/h, Q1 - 6 mm/h, Q3 - 24 mm/h for MCV >100 fL) for Mean Corpuscular Volume.

DISCUSSION

ESR is a frequently ordered test in pathology laboratories as an indicator of illness[4,12]. Despite being nonspecific, its application has wide implications from diagnosing to monitoring infective, inflammatory, malignant and autoimmune diseases, especially polymyalgia rheumatica and giant cell arteritis [2,26,27]. Westergren method remains the gold standard method for ESR measurement as recommended by ICSH, but it has its own disadvantages like requirement of a larger sample volume, time consuming, resource intensive, lacks controls and calibrators, requires correction with low HCT samples and many external factors like vibrations, tilting and temperature may affect it [1,3,7,10,13,15,21]. To combat these limitations, the manual method is quickly being replaced by automated instruments [13]. Test 1 analyzer is a type of alternate Westergren method [14] with a working principle of capillary photometry kinetic technology [1,10]. It uses samples with EDTA anticoagulant, is safer for lab personnel and gives results quickly, especially for a larger sample load with a throughput of at least 150 samples/hour [2].

This study aimed to compare the analytical performance of Test 1 analyzer against the Westergren method amongst various subsets of demographic data, ESR values, Hb ranges, HCT and MCV. Statistical assessment was done using Spearman' rho correlation, Bland Altman analysis and Passing Bablock regression analysis. Results showed good correlation across all age groups and both genders.

Upon analysing the overall ESR values, a good Spearman's correlation of 0.852 [$y = 9.818 + 0.941x$, C.I. - 0.878 to 1.003] was obtained as shown by linear regression and Bland Altman analysis (fig.3 & 4). Similar observations were made by various other studies comparing the two methods [1,17,28]. A higher mean value was observed by automated method as compared to Westergren method, which aligned with the observations of Venapusa et al and Cennamo et al [2,29]. While comparing the subgroups based on ESR values (0 - 20, 21 - 70 and >70 mm/h), we observed a fair correlation amongst the first two categories and a very poor r value of 0.161 (p-value of 0.239) in the third category of ESR >70 mm/h. The results of the group with very high ESR could be limited by a small sample size of only 10.7% in our study [1]. Sonmez et al also compared the Test 1 analyzer with the Westergren method and described concordant findings [10]. Subramanian et al also noted a marked discrepancy in the results for higher ESR values and recommended further studies and the use of correction factor in high range of ESR by automated methods [8]. Chaiwong et al ($r = 0.902$) and Charaniya et al ($r = 0.94$) recorded stronger positive correlation between the two methods [17,28].

Assessing the results according to HCT level showed that there is a good comparability between the two methods with HCT ≥ 35% with $r = 0.845$. The second group with HCT < 35% showed a fair correlation with $r = 0.758$, which improved ($r = 0.779$) after correcting the ESR results obtained by the Westergren method using Fabry's formula. Our data aligned with several other researchers [10,28]. Cha et al also stated that Westergren method overestimated ESR value in samples with low HCT [30]. Test 1 analyzer has an advantage over the Westergren method as it eliminates the step of correction for low HCT levels.

We examined the inference of Hb values in depth by subcategorising according to age and gender for anemia status. We observed positive correlation amongst all groups with relatively weaker r values in groups with severe anemia irrespective of age and gender. Narang et al compared ESR measurement by automated method based on capillary photometry with Westergren method specifically in anemic patients and observed positive correlation, highest in low-ESR range ($r = 0.65$) and least in very high ESR range ($r = 0.18$) [3]. Cennamo et al also compared the Test 1 analyzer against Westergren method in samples with low ranges of Hb ($r = 0.91$) and HCT ($r = 0.92$) and found no significant systemic difference, highlighting that Test 1 automated analyzer is independent of anemic status [2].

We also compared the two methods for the red cell size by analysing the ESR values according to low (<80 fL), normal (80 - 100 fL) and high (>100fL) MCV and found the best correlation in the group with normal MCV ($r = 0.885$, p-value <0.001). Groups with low and high MCV also showed positive correlation of 0.753 (p-value <0.001) and 0.722 (p-value < 0.001), respectively. Iram et al compared an automated ESR analyzer with the Westergren method for various red cell parameters, including Hb, HCT and MCV, and observed positive correlations for all the parameters [13].

CONCULSION

The ESR represents not only the measurement of a single analyte, but the outcome of a complex biophysical process with recognised clinical significance and treatment implications. The automated methods are faster, safer and can efficiently help optimize time and lab resources, especially in high-volume laboratories. This study assessed the analytical validity of an alternate method of automated ESR measurement.

In conclusion, the findings of this study showed a good comparability between Test 1 analyzer and the gold standard Westergren method for ESR estimation. It also performs well with varying ranges of Hb, HCT and MCV. However, samples with very high ESR values measured by automated methods should be interpreted with precaution, and may be corroborated by the Westergren method. Additional studies are warranted in samples with markedly elevated ESR values to compare the results of alternate automated measurement methods with the standard Westergren method.

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