



Evaluation of Erythrocyte Sedimentation Rate by an Automated ESR Analyzer Assuming Manual Westergren Method as Gold Standard at S.M.S. Medical College and Hospital, Jaipur (Rajasthan)

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Abstract

Background: *The erythrocyte sedimentation rate (ESR) is a widely used simple, inexpensive laboratory test often requested in various clinical conditions. Many automated ESR analyzers are being evolved for measuring ESR to improve efficacy.*

Aims and Objective: *To evaluate the performance of the Automated ESR Analyzer and to assess and compare accuracy of ESR readings by Automated ESR.*

Material and Methods: *The 425 samples from 425 consecutive eligible patients fulfilling the inclusion criteria were collected at a tertiary care center in Jaipur and data was analyzed.*

Results: *Bland and Altman analysis revealed low degree of agreement between two methods especially for ESR values > 25 mm/hr. For all the 425 samples, the mean difference and 95% limits of agreement of both the methods were -0.49 ± 10.09 (95% limits of agreement, -10.58 to 9.60). For ESR values > 25 mm/hr, the 95% limits of agreement was -11.52 to 10.05. For ESR values ≤ 25 mm/hr the 95% limits of agreement was -4.78 to 5.17, which showed a very good agreement between both the methods.*

Conclusion: *The Automated Analyzer tend to underestimate the Westergren method ESR values which were ≥ 25 mm/hr. The agreement for ESR value ≤ 25 mm/hr was very good. Hence a correction factor should be applied for ESR values while using Automated Analyzer especially for higher ESR values.*

Keywords: *Comparison, ESR, Westergren, Automated.*

Introduction

The erythrocyte sedimentation rate (ESR) is a simple and inexpensive laboratory test for assessing the acute phase response. ESR is widely used in clinical practice as an indicator of inflammation, infection, trauma or malignant disease.^[1] It can be effective for determination of prognosis or monitoring the disease activity,

response to therapy and even in the diagnosis of certain clinical condition.^[2]

ESR is often preferred by the clinician in the requisition form along with complete blood counts (CBC) and peripheral blood film (PBF).^[3]

ICSH recommended the westergren method for measuring ESR as the method of choice.^[4,5]

Westergren method mostly use the sedimentation

principle in the original westergren pipette or vacuum tube to measure the ESR as the distance that the column of blood cells falls in one hour.^[6,7] It is expressed in milli meters per hour. It varies between age groups, sexes and disease conditions. Despite of many advantages the risk of contact with blood specimen is very high and it is time consuming, modifications in the reference method were made and ICSH guidelines now allow for the use of alternative ESR techniques provided that comparability with the Westergren method is achieved.^[8]

Over the last few years many newer and safer methods have evolved to determine ESR accurately without added risk.^[9] They also use less amount of blood sample.^[1,10]

The automated ESR analyzer in our laboratory gives ESR readings of 150 samples in 1 hour. The principle of measuring is photometrical capillary stopped flow kinetic analysis.

The study was carried out with the aim to evaluate the performance of the Automated ESR Analyser used in our laboratory to assess and compare the results with Manual Westergren Method as Gold standard.

Materials and Method

The study was performed in the Central Laboratory, Department of Pathology, SMS Medical College and Hospital, Jaipur, Rajasthan. It was Laboratory based, Cross- sectional, descriptive type of observational study, started after approval from ethical committee of institution and performed from October 2018 to November 2019.

Inclusion Criteria

- 1) Patients from both sexes and all age groups.
- 2) Patients with hematocrit more than or equal to 30% and less than or equal to 36%.

Exclusion Criteria

- 1) Blood collected by vein puncture taking more than 30 seconds and with excessive venous stasis was excluded

- 2) Blood samples which were not in proper proportions to the anticoagulant, strongly lipidemic, hyperbilirubinemic, hemolyzed were excluded.
- 3) Blood samples having hematocrit less than 30% and more than 36%.

The 425 samples from 425 consecutive eligible patients fulfilling the inclusion criteria were selected out of random blood samples received in the laboratory.

Sample Collection

Whole blood samples were drawn from antecubital vein from arm using 5 ml syringe within 30 seconds and two EDTA vials were used to collect blood (2 ml in each vial).

Westergren Method

In this method, a disposable plastic tube with a bore size of 2.55 mm and a length of 230 mm (Westergren pipette), vertically aligned, open at both ends is used. The pipette is filled with K3 EDTA anticoagulated venous blood to a height of at least 200 mm. The sedimentation occurring at 60 minutes from beginning of the test is noted in mm/hr.

Automated Method

Automated ESR Analyzer used in our laboratory is a fully automated analyzer with a photometrical capillary stopped flow kinetic analysis. It uses EDTA anticoagulated blood samples. A minimum of 800 microlitres of blood is required. 60 samples can be run in one time. First result is available after 4.4 minutes of mixing and 20 seconds of processing and after that every 20 seconds we get results. 60 samples are processed in 24 minutes (150 in 60 minutes).

Observations and Results

All the 425 samples included in our study were within the recommended ICSH hematocrit range ($\leq 36\%$ and $\geq 30\%$). The ESR values obtained by both the methods were compared and analyzed by using Pearson's Correlation graph and Bland and Altman analysis.

Table 1: Correlation between ESR values by Westergren and Automated Analyzer method

Parameters	Pearson's Correlation coefficient (r)	P-Value
ESR	0.991	< 0.001

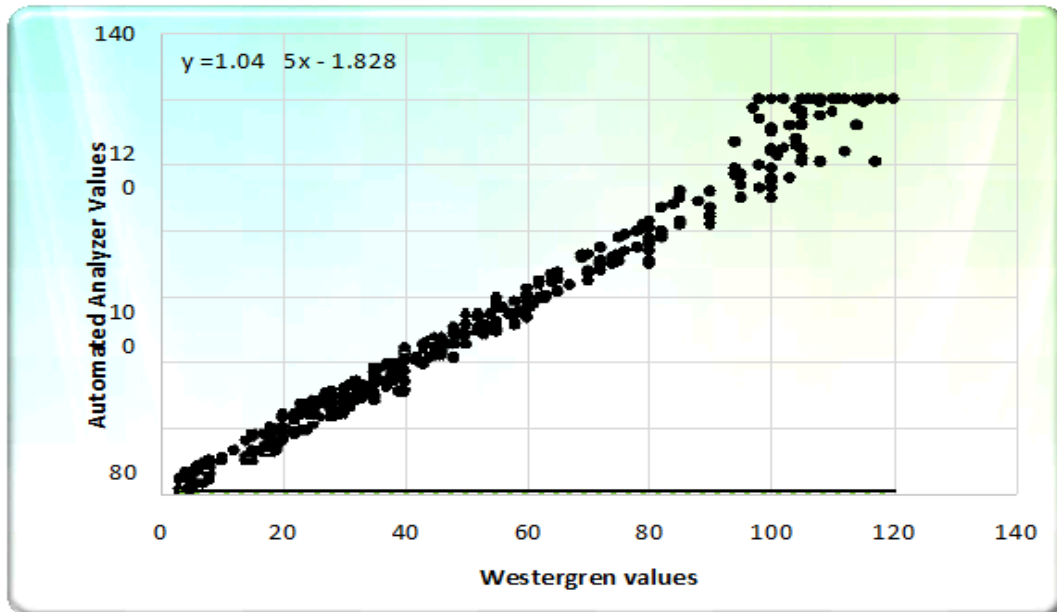


Fig. 1: Correlation of ESR by Westergren and Automated Analyzer method

ESR values by Automated Analyzer showed a positive correlation with ESR values by Westergren Method, that was statistically highly significant (P<0.001). The Pearson's correlation

coefficient 'r' was 0.991 established the strong positive correlation between two parameters. The Pearson's correlation equation was: $Y = 1.0458x - 1.8281$.

Table 2: Mean difference in ESR values as measured by Westergren and Automated Analyzer method

Mean Difference	SD	95% Limits of Agreement
-0.489	5.147	-10.579 to 9.600

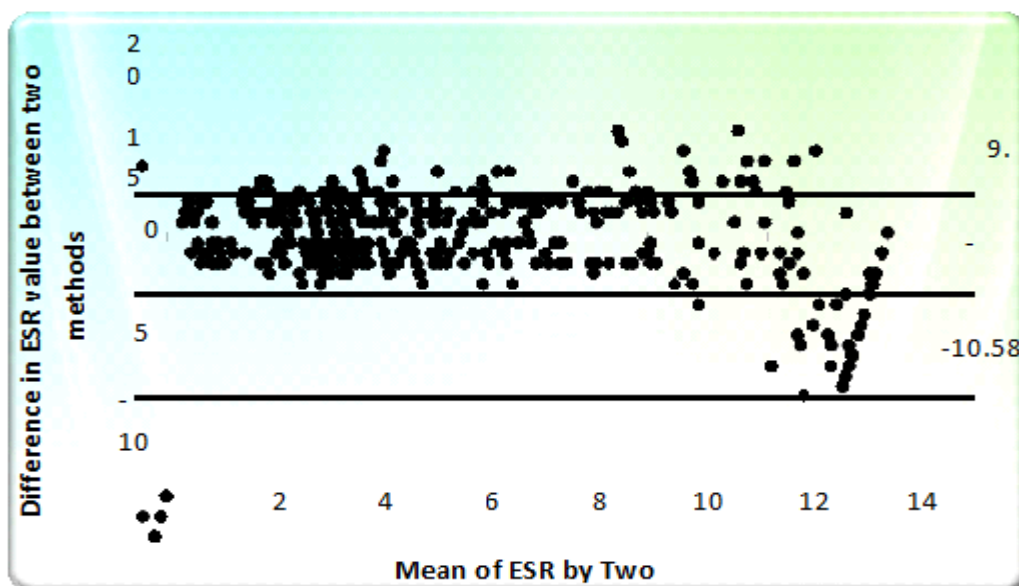


Fig.2: Bland and Altman analysis of the comparison between Westergren method and Automated method, mean difference -0.49; 95% limits of agreement are from -10.58 to 9.60.

The mean of ESR values by both the methods were plotted against difference between ESR values of both the methods. The mean difference between the ESR values by two methods and 95% limits of agreement was -0.49 ± 10.09 (95% limits of agreement, -10.58 to 9.60). The ESR readings for 95% of subjects as measured by the Automated Analyzer will be 10.58 mm/hr below

the Manual Westergren Method or 9.6 mm/hr above it. There is some discrepancy between ESR values by both the methods.

Table 3: Mean difference in ESR readings as measured by Westergren and Automated Analyzer method for ESR values ≤ 25 mm/hr

Mean Difference	SD	95% Limits of Agreement
0.195	2.542	-4.788 to 5.177

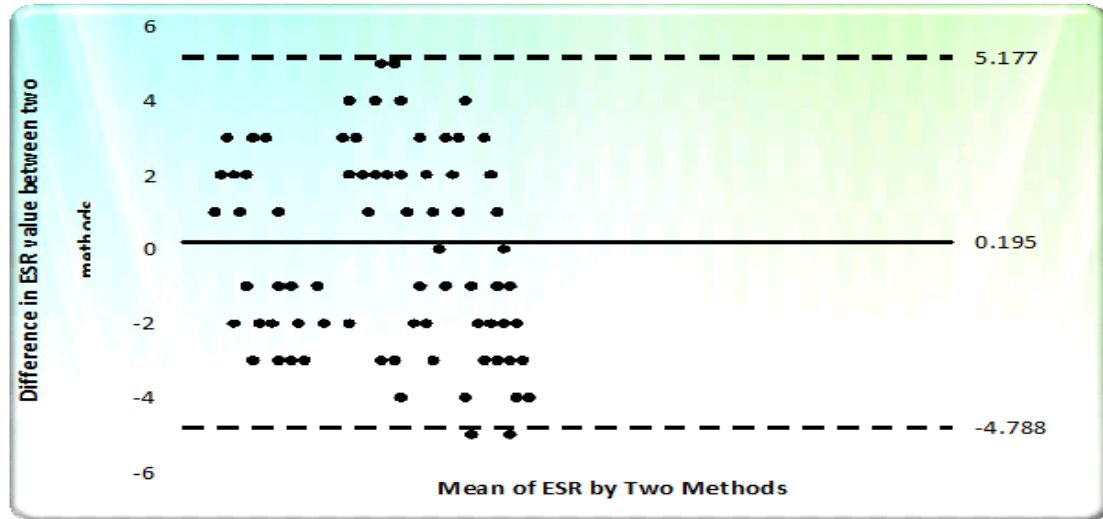


Fig. 3: Bland and Altman analysis of the comparison between Westergren and Automated method for ESR values ≤ 25 mm/hr

The Agreement between the ESR values (≤ 25 mm/hr) measured by both methods was very good with mean difference and 95% limits of agreement were 0.195 ± 4.982 (95% limits of agreement, -4.78 to 5.17).

Table 4:- Mean difference in ESR readings as measured by Westergren and Automated Analyzer method for ESR values > 25 mm/hr

Mean Difference	SD	95% Limits of Agreement
-0.737	5.502	-11.521 to 10.047

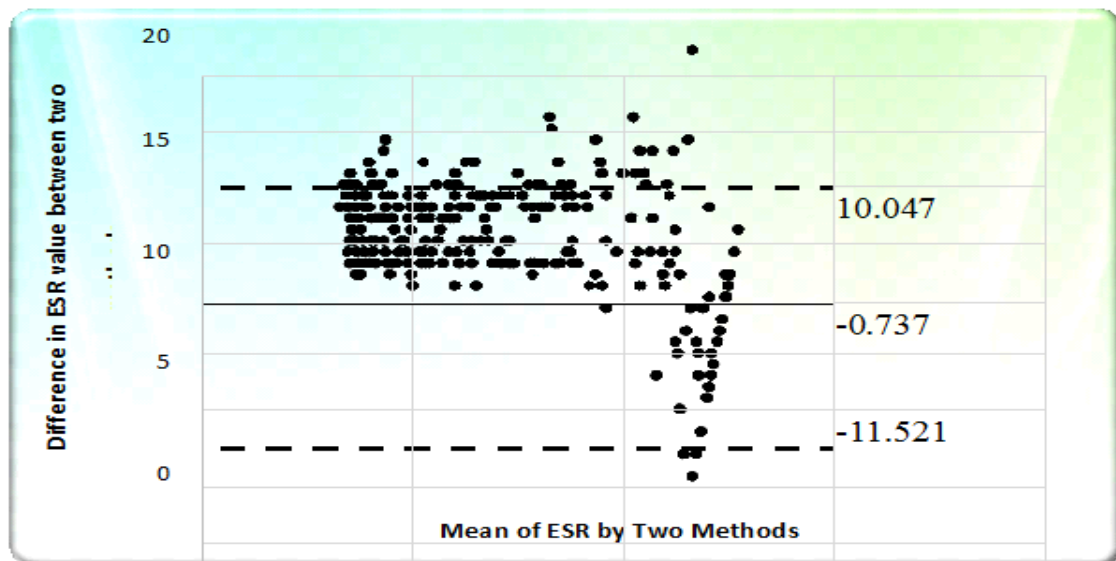


Fig. 4: Bland and Altman analysis of the comparison between Westergren and Automated method for ESR values > 25 mm/hr

For ESR values > 25 mm/hr, the mean difference between ESR values by both methods and 95% limits of agreement were -0.737 ± 10.783 (95% limits of agreement, -11.52 to 10.05).

Discussion

One of the oldest clinical laboratory methods and one that has not been changed over years is the Westergren ESR procedure. The erythrocyte sedimentation rate is a relatively simple and inexpensive test used to assess patients with acute or chronic inflammatory processes.^[8,11] It serves as a useful aid in the diagnosis of various clinical conditions, and has been shown to correlate with an unfavourable prognosis in the neoplastic disease and coronary artery disease.^[12]

The present study was conducted in SMS Medical College, Jaipur (Rajasthan) during a period of 14

months. Total 425 samples of random individuals were collected which included, 295 female subjects and 130 male subjects. 113 subjects showed ESR values ≤ 25 mm/hr and 312 subjects showed ESR values > 25 mm/hr.

It was observed that, ESR values measured by Automated ESR Analyzer showed a positive correlation with ESR values measured by Manual Westergren Method, that is statistically highly significant ($p < 0.001$). The Pearson's correlation coefficient 'r' was 0.991, establishing the strong positive correlation between two parameters.

Many of the other studies also showed a positive correlation of ESR values measured by Automated Analyzer and Manual Westergren Method, like AlFadhli et al,^[13] Hardeman et al,^[14] Asif et al,^[15] Hashemi et al,^[16] Vennapusa et al^[4] and Sonmez et al.^[17]

Table 5: Comparison of correlation between ESR values by Manual Westergren Method and Automated Analyzer in present study with other studies

S.N.	Study	Pearson's Correlation Coefficient (r)	P value
1.	AlFadhli SM et al, ^[13] 2005	0.91	<0.0001
2.	Hardeman MR et al, ^[14] 2009	0.90	<0.0001
3.	Asif N et al, ^[15] 2012	0.97	0.000
4.	Hashemi R et al, ^[16] 2015	0.98	<0.001
5.	Vennapusa B et al, ^[4] 2015	0.95	<0.0001
6.	Sonmez C et al, ^[17] 2017	0.77	<0.001
7.	Present Study, 2019	0.99	<0.001

The Pearson's Correlation Coefficient 'r' measures a relation between two variables, not the agreement between them. A perfect agreement will only be found if all the points in the Pearson's correlation coefficient graph lie along the line of equity, but a perfect correlation is found if the points lie along any straight line. Therefore, the calculation of 'r' is not sufficient and could be misleading since the strong correlation does not show the agreement between the two measurement.^[13] Agreement analysis is more sensitive method than the correlation coefficient for comparison between the two methods.

Since a high correlation does not prove the agreement between the two measurements, the Bland and Altman statistical method was used to measure the limits of agreement of the two measurements.^[18] The difference (Westergren

method-Automated method) between ESR values measured by Manual Westergren Method and Automated Analyzer were plotted on 'Y' axis and mean of ESR values measured by both the methods were plotted on 'X' axis. Then the limits of agreement were calculated as $d \pm 1.96SD$, where 'd' is the mean of the differences between the ESR values measured by the Manual Westergren Method and Automated Analyzer and 'SD' is the standard deviation of the differences.

Bland and Altman analysis revealed mild variation between two methods especially for ESR values > 25 mm/hr. For all the 425 samples, the mean difference and 95% limits of agreement of both the methods were -0.49 ± 10.09 (95% limits of agreement, -10.58 to 9.60). For ESR values > 25 mm/hr, the 95% limits of agreement was -11.52 to 10.05. For ESR values ≤ 25 mm/hr the 95% limits

of agreement was -4.78 to 5.17, which showed a very good agreement between both the methods.

Table 6: Comparison of mean difference and 95% limits of agreement of present study with other studies.

S. N.	Study	Mean Difference of whole data	95% limits of agreement for whole data	Mean Difference of ESR values >25 mm/hr	95% limits of agreement for ESR values >25 mm/hr	Mean Difference of ESR values ≤25 mm/hr	95% limits of agreement for ESR values ≤25 mm/hr
1.	AlFadhli et.al, ^[13] 2005	-13.18	-37.88 to 11.52	-21.4	-45.2 to 2.26	-3.9	-13.5 to 5.7
2.	Subramanian et.al, ^[19] 2011	-11.2	-46.3 to 23.9	-13.4	-57.3 to 30.5	-7.7	-18.9 to 3.5
3.	Patil et al, ^[9] 2013	-1.01	-44 to 63	-0.44	-1.23 to 40.37	-1.73	-18 to 10
4.	Dhruva et.al, ^[20] 2014	2.37	-13.94 to 18.68	2.58	-15.39 to 20.55	1.22	-2.50 to 4.94
5.	Present study, 2019	-0.49	-10.58 to 9.60	-0.737	-11.52 to 10.05	0.195	-4.78 to 5.17

Previous various other studies including Al Fadhli et al^[13], Subramanian et al^[19], Patil et al^[9], and Dhruva et al^[20], also showed such discrepancies for ESR values > 25 mm/hr but a good agreement for ESR values ≤ 25 mm/hr between Manual Westergren Method and Automated Analyzer.

We found that in various studies, the agreement between Automated Analyzer and Manual Westergren Method for lower ESR values was found to be very good but the agreement between both methods for higher ESR values was poor. So the Automated Analyzers must be validated with Manual Westergren Method to achieve accuracy according to ICSH guidelines. If required a correction factor should be applied to achieve agreement between both the methods.

Conclusion

We concluded that the Automated Analyzer showed a very good agreement with Manual Westergren Method for ESR values ≤25 mm/hr, However the Automated Analyzer tend to underestimate the Westergren ESR value ≥ 25 mm/hr. So a correction factor should be applied for ESR values when using this Automated Analyzer especially for ≥ 25 mm/hr values, as this will increase agreement with gold standard Manual Westergren Method. Once validation with gold standard Manual Westergren Method is achieved Automated Analyzer will improve efficacy, reduce human efforts, quick results will be available and will reduce risk of blood borne

infections to medical personnels.

Conflict of Interest: None

Source of Support: Nil

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