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# Comparison of Automated Erythrocyte Sedimentation Rate with Gold Standard Westergren Method and its Correlation with Red Cell Parameters

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## Abstract

**Objective:** To compare the automated ESR with the gold standard Westergren method and investigate its correlation with red cell parameters.
**Methodology;** A total of 400 randomly collected blood samples (15-78 years of age) from Armed Forces Institute of Pathology (AFIP) Rawalpindi were assayed parallelly using Westergren method and Greiner-Bio-One (Vacuette®) SRS 20/II ESR analyzer from October 2022 to January 2023. Results of these assays were analyzed using the Kolmogorov-Smirnov test for normality and Spearman's correlation test for correlation.
**Results:** This study revealed a strong and significant correlation between the automated ESR analyzer and the classical gold standard Westergren method ($r=0.907$; $p < 0.001$). Additionally, the red blood cell parameters showed a positive and significant correlation with both automated and Westergren methods ($p < 0.001$).
**Conclusion:** With strong correlation between Greiner-Bio-One (Vacuette®) SRS 20/II ESR automated analyzer and the reference Westergren method, this study concludes that the automated technique is substantially associated with the Westergren method for estimating ESR, and red blood cell parameters have a positive and significant correlation with both methods.
**Keywords:** Erythrocyte sedimentation rate, Westergren methods, red cell parameters, automated method

## Introduction

ESR (Erythrocyte sedimentation rate) is a simple, cost effective and frequently requested laboratory test that is used as an indicator of inflammation, infections and malignancies. In cases of certain diseases like rheumatoid arthritis, polymyalgia rheumatica, giant cell arteritis and multiple myeloma, ESR has a central role in diagnosis and disease progress monitoring.[1, 2]

Westergren ESR method is simple and cost effective but it is time consuming, labor intensive and affected by diverse environmental and physiological factors such as temperature, tilting of the tube and paraproteinemia. Over the past two decades, modified and alternate methods of ESR measurement have been introduced. These techniques aimed to reduce the flaws of Westergren method while keeping its benefits. In the past many studies have been conducted on automated high throughput ESR techniques based on different principles of ESR measurement using different anticoagulants.[3]

These techniques are now widely used for their benefits of reduced report turnaround time, with decreased exposure and dealing with blood samples. International council for standardization in haematology (ICSH) recommends evaluation of the alternate methods before implementation in clinical laboratories.[4,5]

ESR values of up to 15 mm/h in men and up to 20 mm/h in women are considered normal.[6] Though ethylenediaminetetraacetic acid (EDTA) samples are suitable for both hematology and ESR measurements, diluted specimens with citrate in a 1:4 ratio are commonly preferred for ESR analysis.[7]

The International Council for Standardization in Hematology (ICSH) has developed standards for measuring ESR[5], and Thomas *et al.*[8] have provided

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recommendations for validating and calibrating ESR method. Because of the changes in the blood sample quality (citrate or EDTA, sampling tubes), measuring times, and measuring principles, ESR calibration is critical for reliable readings. Dr. A Westergren and Dr. R Fahraeus described the ESR technique for the first time in 1921.[9] Traditional manual techniques primarily rely on the principle of sedimentation to determine the ESR (erythrocyte sedimentation rate). To carry out this procedure, the distance that a group of blood cells descends in one hour is measured using either the original Westergren pipette or a vacuum tube. In order to address the practical limitations of the original Westergren ESR method, various automated techniques were developed.

These approaches assess the ESR by measuring the sedimentation of red blood cells in specialized tubes, after diluting whole blood with EDTA or citrate. The recorded sedimentation (in millimeters) is then converted to Westergren units (millimeters per hour). The main benefit of these methods, in comparison to the manual Westergren-based method, lies in their use of a completely enclosed, automated system that yields more readily accessible results.[10]

ESR can be influenced by two key elements; factors directly associated with erythrocytes include their size and count, while the others include fibrinogen and immunoglobulins.[11]Nevertheless, there is limited research exploring the impact of red blood cell parameters on ESR.

The aim of this study is to compare the automated erythrocyte sedimentation rate with gold standard Westergren method and its correlation with red cell parameters.

## Methodology

This correlation study was performed at department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from October 2022 to January 2023. Approval was taken from Institutional Review Board (IRB), vide reference number FC-HEM22-23/READ-IRB/23/1984.

Sample size of 384 was calculated by WHO calculator using the formula $n = z\ 2\ /4e\ 2$. Where $n$= sample size and $e$= acceptable sampling error ($e$= 0.05), This formula can be used when population and/or prevalence are unknown.[12]

Sampling was done using a non-probability consecutive sampling technique. After informed written consent demographic data was obtained. We included 400 blood samples from adult patients (15-78 years of age) presented at AFIP for evaluation of ESR and RBC parameters. Hemolyzed, clotted and lipemic blood samples were excluded.

Manual Westergren method was performed as gold standard reference method. K2 EDTA blood samples were analyzed at room temperature (18-25$^0$C) within 4 hours after collection according to the ICSH recommendations. EDTA samples were mixed with 3.8% trisodium citrate (in a ratio of 4:1) manually. Samples were aspirated immediately in glass Westergren tubes (overall length of 300 mm, internal diameter of 2.55 mm and scale graduated over the lower 200 mm) that were placed vertically in supporting rack. After one-hour results were determined visually as the distance from the bottom of the surface meniscus to the top of the column of sedimenting erythrocytes.[13]

SRS 20/II (Greiener-Bio-One) was used as automated method of Westergren using solid plastic evacuated (Vacuette®) tubes. These evacuated blood collection tubes (9 mm in diameter with height of 120 mm) are polypropylene coated with brom butyl rubber caps that aspirate 1.6 ml of whole blood in trisodium citrate in a ratio of 4:1. This analyzer performs the ESR test in 15 minutes and 30 minutes giving a final result in mm/hour, comparable to 1 hour gold standard manual Westergren method readings. The automated analyzer has twenty independent channels for ESR measurement. Temperature adjustments are maintained at 18$^o$C and using infrared beams each channel reads the sample randomly.

RBC parameters (Hb, RBC count, Hct, MCV, MCH, MCHC and RDW) were obtained using Sysmex XN 1000 analyzer. Participants of this study were split in two categories on the basis of age, group (1) 15–25 years and group (2) 26–78 years. CBC parameters were categorized in three groups for each parameter i.e., MCV: (1) normocytic: 76-96 fl, (2) macrocytic: >96 fl and (3) microcytic: <76 fl; MCHC: (1) normochromic :31.5– 34.5%, (2) hyperchromic >34.5% and (3) hypochromic <31.5%; hemoglobin: (1) normal:13–17 g/dL), (2) low <13 g/dL, and (3) raised >17 g/dL.

Data analysis was done on SPSS version 25.0. To determine whether the data adheres to a normal distribution, the Kolmogorov-Smirnov test was conducted. In cases where the data does not conform to a normal distribution, the Spearman's correlation test was utilized, and the median and interquartile range (IQR) was calculated. A significance level of $p \leq 0.05$ was deemed significant.

## Results

A total of 400 patients were included in this study. Median age was 38.0 (47.0 – 27.0) Years ranging from 15 to 78 years. Among them 276 (69.0%) were male and 124 (31.0%) were females. The Westergren method yielded measurements of the ESR ranging from 2 mm/1st hour to 130 mm/1st hour, with a median of 31.0 (39.0 - 15.0) mm/1st hour. Automated method produced ESR measurements ranging from 2 mm/1st hour to 121 mm/1st hour, with a median of 32.0 (43.0 - 16.0) mm/1st hour. Notably, there was a strong and significant correlation ($r=0.907$; $p < 0.001$), as depicted in Figure 1, between the automated method and the Westergren method when determining the ESR.

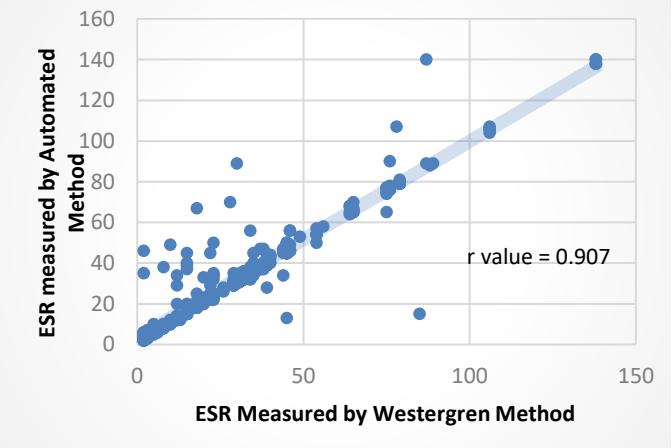


Figure 1. Relationship between Automated and Westergren ESR. (n=400)

Table I demonstrates a statistically significant correlation between automated SRS 20/II analyzer and the manual gold standard Westergren method in relation to age and gender for the ESR values obtained. Similarly, for the red cell parameters of our study, ESR values obtained by automated SRS 20/II analyzer and Manual gold standard Westergren method found to have positive strong correlation with highly significant p value < 0.001.

Table I: Correlation between Automated and Westergren ESR with respect to Age and Gender Distribution. (n=400)

| Parameters | | Westergren Median (IQR) | Automated Median (IQR) | Correlation |
|---|---|---|---|---|
| Age in Years | 15 – 25 (n=69) | 32.0 (37.5 – 10.0) | 32.0 (38.0 – 13.0) | 0.878** |
| | 26 – 78 (n=331) | 30.0 (39.0 – 15.0) | 32.0 (45.0 – 17.0) | 0.912** |
| Gender | Male (n=276) | 30.0 (39.8 – 15.0) | 32.0 (45.0 – 16.3) | 0.878 |
| | Female (n=124) | 30.0 (38.8 – 15.0) | 32.0 (39.8 – 16.0) | 0.877 |

***Correlation Significant at 0.001 level*

Table II: Correlation between Automated and Westergren ESR for Red Cell Parameters. (n=400)

| Parameters | | Westergren Median (IQR) | Automated Median (IQR) | Correlation |
|---|---|---|---|---|
| MCV | Microcytic (n=148) | 32.0 (38.0 – 15.0) | 34.00 (42.3 – 21.2) | 0.849** |
| | Normocytic (n=210) | 31.0 (40.0 – 15.0) | 32.0 (44.3 – 15.7) | 0.925** |
| | Macrocytic (n=42) | 26.0 (41.2 – 12.8) | 29.5 (42.7 – 14.8) | 0.991** |
| MCHC | Hypochromic (n=123) | 32.0 (40.0 – 18.0) | 32.0 (45.0 – 18.0) | 0.887** |
| | Normochromic (n=178) | 29.0 (38.0 – 15.0) | 32.0 (42.5 – 16.0) | 0.875** |
| | Hyperchromic (n=99) | 32.0 (29.0 – 15.0) | 32.0 (43.0 – 15.0) | 0.976** |
| HB | Low (n=165) | 32.0 (39.0 – 18.0) | 32.0 (40.0 – 17.0) | o.970** |
| | Normal (n=209) | 29.0 (37.5 – 15.0) | 32.0 (41.5 – 16.0) | 0.854** |
| | High (n=26) | 28.0 (50.7 – 14.3) | 35.5 (53.7 – 14.3) | 0.968** |
| RBC | Low (n=87) | 32.0 (45.0 – 15.0) | 34.0 (47.0 – 21.0) | 0.927** |
| | Normal (n=268) | 31.0 (39.0 – 15.0) | 32.0 (43.0 – 17.0) | 0.895** |
| | High (n=45) | 18.0 (34.0 – 10.0) | 23.0 (36.5 – 11.0) | 0.906** |
| HCT | Low (n=107) | 32.0 (45.0 – 18.0) | 33.0 (49.0 – 20.0) | 0.983** |
| | Normal (n=203) | 32.0 (38.0 – 15.0) | 32.0 (39.0 – 17.0) | 0.883** |
| | High (n=90) | 29.0 (37.0 – 12.7) | 31.0 (38.3 – 15.0) | 0.835** |
| RDW | Low (n=139) | 32.0 (40.0 – 15.0) | 32.0 (42.0 – 16.0) | 0.984** |
| | Normal (n=187) | 30.0 (38.0 – 15.0) | 32.0 (44.0 – 17.0) | 0.839** |
| | High (n=74) | 31.0 (44.3 – 15.0) | 32.0 (44.3 – 15.0) | 0.949** |

## Discussion

The Westergren method is simple, cost effective and, despite limitations, frequently requested test. According to ICSH it is the reference method for ESR measurement.[8] In recent decades several new techniques have been developed to overcome the shortcomings of manual Westergren ESR method and automated systems for ESR measurement have been introduced. They use either diluted or undiluted blood samples. Modifications include simplified usage, time efficiency, closed sample manipulation, vacuum-controlled sample aspiration (to ensure proper dilution with the anticoagulant), and automated mixing. Another advantage of automated techniques is their ability to safeguard against external influences such as dust particles, temperature, diluent ratios, and tube position, which may affect the final reading. Additionally, this method allows for a higher volume of sample evaluation within a specified timeframe compared to the manual method, which is particularly advantageous for tertiary care hospitals with heavy workloads. However, to substitute the traditional ESR method, it is necessary to verify the reliability of these automated devices by comparing them to the Westergren method. [14,15]

Findings of current study indicate a robust and statistically significant association between the Westergren method and the automated technique for measuring ESR ($r = 0.907$; $p < 0.001$). Previously *Asif et al*[16] found a substantial and strong relationship between automated and Westergren methods (r=0.97, p= 0.00). Hashemi *et al* in 2015[17], Preet *et al* in 2018[18] and Drashti *et al* in 2016[19] also reported similar results as in our study (r=0.987; p<0.001).

In 2014 Sönmez *et al*. (r=0.978; p<0.05)[20] in 2001 Wiwanitkit *et al*. (r=0.98; p<0.05)[21] and in 2011 Cerutti *et al*. (r=0.816, p<0.05)[22] also found similar association results between values of these two techniques. In 2010, Horsti and colleagues reported a relatively lower association (r=0.72; p<0.01) between these two techniques.[23] Venapusa[24] conducted a study that indicated a 95% correlation between the automated and Westergren methods. However, he also observed higher ESR values with the automated method. He couldn't pinpoint the exact type of systemic bias, but he believed it was responsible for these findings. Al Fadhli discovered notable variations in measurements when comparing the reference and automated techniques, particularly with higher ESR readings. However, when it came to normal and slightly elevated ESR values, both techniques produced comparable results.[25]

Our study also found a positive correlation of all blood parameters with automated and Westergren methods with p value < 0.001. No previous studies were found similar to this comparison.

LIMITATIONS OF THE STUDY: Large sample size with particular consideration of high ESR values can help accurately analyze the discrepancies between Westergren and automated techniques. It is plausible to propose that the automated approach is reliable and appropriate for routine ESR measurements. To enhance the credibility of the outcomes obtained from the automated analyzer, further validation experiments and studies would be required.

## Conclusion

The current study found a substantial association between the Westergren technique and the automated technique of estimating ESR. Our study also concluded that there was a positive significant correlation of all blood parameters with automated and Westergren methods with p value < 0.001.

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