

## ORIGINAL ARTICLE

**Comparison of Erythrocyte Sedimentation Rate by Vision Principle and Westergren Method**AAYZA MUJAHID<sup>1\*</sup>, JAWAD ZAFFAR CHOUDRY<sup>1</sup>, HAMID IQBAL<sup>2</sup>, SYEDA BINT-E-ZAHRA<sup>1</sup>, SAADIA LATIF<sup>1</sup>, ZAHRA HABIB<sup>3</sup><sup>1</sup>Department of Hematology, Combined Military Hospital, Multan-Pakistan<sup>2</sup>Department of Hematology, Combined Military Hospital, Quetta-Pakistan<sup>3</sup>Department of Physiology, Multan Medical and Dental College, Multan-PakistanCorrespondence to Dr. Aayza Mujahid, Email: [aayzamujaahid.am@gmail.com](mailto:aayzamujaahid.am@gmail.com). Tel:+92-316-8050786**ABSTRACT****Background:** Erythrocyte sedimentation rate is a common test used as an assessment tool for inflammation since many years.**Aim:** To compare erythrocyte sedimentation rate by automated ESR analyzer working on vision principle against conventional manual Westergren method.**Study design:** Cross sectional study.**Methodology:** A total number of 120 samples were taken from routine hospital patients whom ESR was requested through non-probability consecutive sampling following ethical approval. Samples were subjected to ESR estimation by manual Westergren and automated methods. Data was analyzed by SPSS version 22. Independent sample t-test used to compare erythrocyte sedimentation rate by automated ESR analyzer working on vision principle against conventional manual Westergren method with p-value  $\leq 0.05$  as significant. Pearson correlation was also applied.**Results:** Mean  $\pm$  SD for Manual Westergren method was  $39.37 \pm 25.486$  and Automated Vision principle was  $25.83 \pm 21.015$ . Levene's Test for Equality of Variances have p-values for ESR in patients with Manual Westergren method & Automated Vision principle was 0.018 with F-value 5.670. There was a non-linear relationship between the two methods (P value < 0.01).**Practical Implication:** This study helped in exploring reliability of different ESR estimation methods as ESR results have notable impact on patient diagnosis and follow-up. With increasing demands and burden of patients in labs, new reliable and quick techniques for ESR determination are need of hour.**Conclusion:** It was concluded that automated methods showed good correlation to manual Westergren method thus are reliable for ESR estimation. However, manual Westergren remained gold standard procedure for ESR estimation.**Keywords:** Erythrocyte Sedimentation Rate, Reliability, Westergren Method and Automated Method.**INTRODUCTION**

Erythrocyte sedimentation rate is a common test used as an assessment tool for inflammation since many years to date<sup>1</sup>. This is an inflammatory marker that gets raised in many infectious conditions and health insults like infarctions, malignancies and autoimmune diseases<sup>2</sup>. According to literature review it is an acute phase reactant<sup>3-5</sup>. Previous studies have shown that ESR differs from other inflammatory markers like rapidly changing C-reactive protein. However, these both markers play important and different roles in diagnosis, management and follow-up of a patient for disease progression. It has been demonstrated previously that ESR is a more reliable and sensitive prognostic tool for chronic inflammatory illnesses like atherosclerosis<sup>6,7</sup>.

It has been seen that its measurement is cheap and easy thus making it a common investigation in almost every clinical laboratories of various sizes. According to previous studies, there are various methods to measure ESR levels while using basic principles. However, all methods need evaluation and validation in reference with standardized method to get synchronization and uniformity for normal values of ESR<sup>8-10</sup>.

According to previous work, samples taken in EDTA tubes are used for both ESR and hematology measurements. However, special medium like 1:5 citrate diluted samples are preferable for ESR analysis and calculation. According to ICSH, Westergren method for ESR measurement has been used as gold standard<sup>11,12</sup>. Other all new methods are validated and calibrated in accordance to Westergren according to guidelines. Its calibration is very important for accurate measurements of ESR due to factors like differences in sampling quality and measuring times<sup>13</sup>. Manual Westergren method is time consuming in comparison to new techniques like automate analyzer.

With advancement and modernization in field of investigation, there happened the development of new methods like automated ESR analyzers for laboratory work. Other factors that have contributed to it include increased sample volumes in

clinical laboratories. There are many automated methods that use different anticoagulants. According to one study, most common anti-coagulant is EDTA due to its usability in other hematological measurements<sup>14</sup>.

There is a complex relationship between ESR values and diagnosis with management, which is probably not properly understand by many people due to lack of knowledge about benefits of correct labs values. ESR estimation by old methods is time consuming so quick lab reports are required in modern era. Due to lack of research culture and development in our society, we lacked to highlight basic important health issue and their impact on quality of health. This study helped in exploring reliability of different ESR estimation methods as ESR results have notable impact on patient diagnosis and follow-up. With increasing demands and burden of patients in labs, new reliable and quick techniques for ESR determination are need of hour. Hence, the current study was planned to determine the reliability of automated method for ESR estimation.

The objective of the study was to compare erythrocyte sedimentation rate by automated ESR analyzer working on vision principle against conventional manual westergren method.

**METHODOLOGY**

This cross sectional study was carried at Combine Military Hospital-Multan. A total number of 120 samples were taken from routine hospital patients whom ESR was requested through non-probability consecutive sampling following ethical approval. Samples were subjected to ESR estimation by manual westergren and automated methods. Blood (3.0ml) was drawn into EDTA tubes than mixed well before analysis. Each sample was first analyzed by automated analyzer than immediately with the classic Westergren pipette. Co-relation between different methods was done using westergren as standard. Written consent was taken. All information was kept recorded. All baseline information was taken in a specialized profoma. Unwilling participants were left out.

**Statistical analysis:** Data was analyzed by SPSS version 22. Independent sample t-test and Pearson correlation was also

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applied. Quantitative parameters were presented as mean ± SD. P-value ≤ 0.05 is taken significant.

**RESULTS**

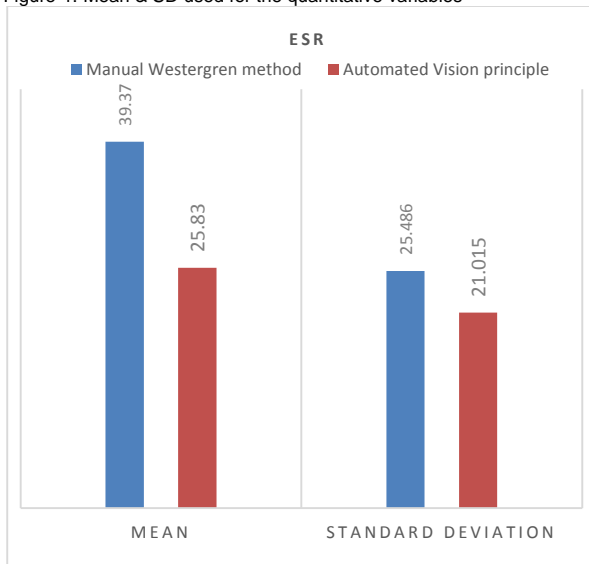
Mean±SD for Manual Westergren method was 39.37±25.486 while Automated Vision principle had mean±SD of 25.83±21.015 as shown in table-1.

Table-1: Quantitative variables as mean ± SD

Variables	Mean	SD
Manual Westergren	39.37	25.486
Automated Vision	25.83	21.015

Mean & standard deviation used for the quantitative variables i.e., Manual Westergren method & Automated Vision principle with graph representation as shown in figure-1.

Figure-1: Mean & SD used for the quantitative variables



Levene's Test for Equality of Variances have p-values for ESR in patients with Manual Westergren method & Automated Vision principle was 0.018 with F-value 5.670 showed all significant p-value was less than 0.05 & concluded that the variance of ESR in patients with Manual Westergren method & Automated Vision principle were significantly not equal as shown by table-2. As Levene's Test for Equality of means showed that some of the means were not equal, so P-value for ESR in patients with Manual Westergren method & Automated Vision principle was 0.000 with t-value 225.801 showed significant p-value as it was less than 0.05 & concludes that the means of ESR of Manual Westergren method & Automated Vision principle is significantly not equal. Moreover, the sign of the mean difference corresponds to the sign of the t-value, the positive t-values indicates that the Mean of ESR in patients with Manual Westergren method was significantly greater than Automated Vision principle.

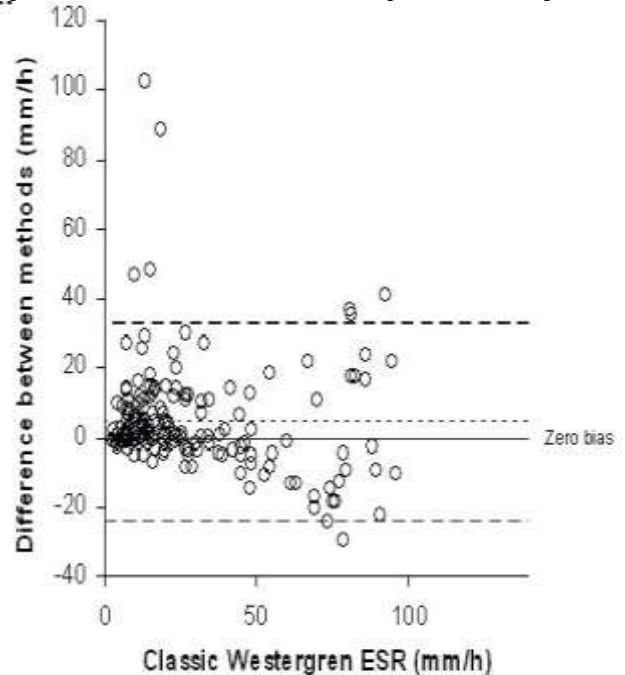
Table-2: Independent Samples Test

ESR_value	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	5.670	.018	4.453	234	.000*	13.542	3.041	7.551	19.533
Equal variances not assumed			4.453	225.801	.000*	13.542	3.041	7.550	19.535

\*Statistically significant

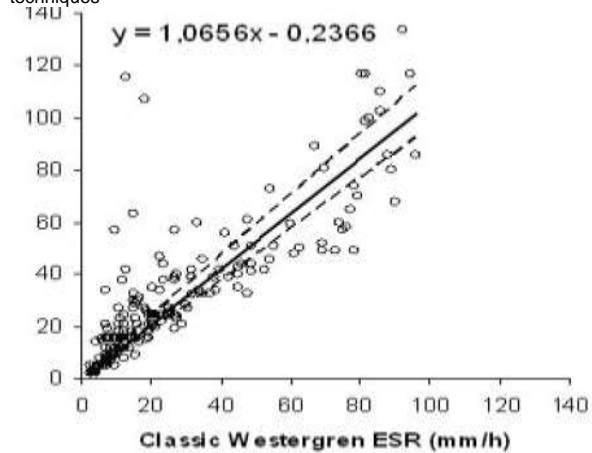
Comparison between different methods for ESR calculation and the classic Westergren method in mm/h (Fig. 2).

Figure-2: Difference between methods according to classic Westergren ESR



Relationship between the two methods was non linear (P value < 0.01) as shown by figure-3.

Figure-3: Relationship between gold standard and automated analyzer techniques



## DISCUSSION

Erythrocyte sedimentation is still a partly understood physicochemical phenomenon of the susceptibility of RBCs to settle down, dependent on the influence of a variety of physiological and patho-physiological factors as revealed by previous literature<sup>15</sup>. The process is characterized by three distinct phases: the aggregation phase characterized by Rouleaux formation followed by settling stage later by packing stage where RBCs are sedimented at the bottom of the tube. Rouleaux formation is crucial for the overall ESR and is largely influenced by the presence of positively charged plasma proteins that induce RBC aggregation, in particular fibrinogen and globulins<sup>16</sup>. Understanding this mechanism was important for the development of novel ESR technologies. While the Westergren method and its modifications simply measure the overall sedimentation phenomenon, alternate methods incorporate measurement principles that assess the erythrocyte sedimentation kinetics mainly in the initial phase and apply measurements at different time intervals<sup>13,15,16</sup>.

According to literature review, method for ESR estimation in laboratory for years is Westergren procedure. With advancement and modernization in field of investigation, there happened the development of new methods like automated ESR analyzers for laboratory work. According to one study, this automated analyzer analyzes almost 135 samples in an hour if the 30 min mode is used. This rapidity and feasibility of this technique attracts and compels laboratory workers to use it as it saves effort and time<sup>14</sup>.

According to literature review, there is slight modification in the technique used in both methods. We followed the methodology as defined by previous researcher with mild modifications<sup>11</sup>. In present study, blood was first drawn into EDTA tube followed by dilution of 4+1 with sodium citrate. This principle clearly deviated from the classic Westergren method. However, Westergren method was more reliable as a reference method for ESR measurements for comparisons because only one anticoagulant is used<sup>12</sup>.

We carried out this study to look for correlation of two automated methods with Westergren method. There was a non-linear relationship between the two methods with significant p-value in our study. Our results were in line with one previous study that showed non-linear relation between standard and automated procedures for ESR calculation<sup>13</sup>. However, another previous study, showed that automated methods though different from Westergren in terms of anti-coagulants used have strong positive linear co-relation thus were paradoxical to present study<sup>14</sup>.

According to our results, Westergren method remained superior to automated methods for ESR estimation though it is more time consuming and requires more hand work. These findings were in line with many other previous studies<sup>13,15,17</sup>.

Hemolysis in the studied range showed to have a negative effect on ESR values obtained by all methods, with a statistically significant decrease observed for automated and the Westergren method. Our approach for hemolysis interference assessment was similar to the ones reported in two previous studies<sup>18,19</sup> that was based on spiking of native samples with a lysing solution that contains glycerol. Equally to our study, it was shown that hemolysis significantly decreases ESR levels when measured by automated technique<sup>19</sup>.

**Limitations:** Small sample size followed by single centre study. Financial constrains added to limitations of study.

## CONCLUSION

It was concluded that automated methods showed good correlation to manual Westergren method thus are reliable for ESR estimation. However, manual Westergren remained gold standard procedure for ESR estimation.

**Authors' Contribution: AM&JZC:** Conceptualized the study and formulated the initial draft, **HI& SBZ:** Contributed to the analysis of data and proofread the draft, **SL& ZH:** Collected data.

**Conflict of interest:** Noting to declare

**Conflict of interest:** None

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