

# COMPARATIVE ANALYSIS OF ERYTHROCYTE SEDIMENTATION RATE MEASURED BY AUTOMATED ANALYSER (ALI FAX ROLLER 20LC) AND WESTERGREN METHOD

Lakshmi Sowjanya Annepu<sup>1</sup>, Aluri Anjana Priyanka<sup>2</sup>, Vutukuru Venkata Sushma<sup>3</sup>, Gudeli Vahini<sup>4</sup>

<sup>1</sup>Final year Post graduate resident, Department of Pathology, ASRAM Medical College, Eluru, A.P, India, Contact number :8985626040

<sup>2</sup>Associate Professor, Department of Pathology, ASRAM Medical College, Eluru, A.P, India, Contact number: 9493376645

<sup>3</sup>Assistant Professor, Department of Pathology, ASRAM Medical College, Eluru, A.P, India, Contact number: 9704345451

<sup>4</sup>Professor and Head, Department of Pathology, ASRAM Medical College, Eluru, A.P, India.

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**Corresponding Author:** Dr Gudeli Vahini 4, Professor and Head, Department of Pathology, ASRAM Medical College, Eluru, A.P, India.

Email: [gudelivahini@gmail.com](mailto:gudelivahini@gmail.com)

## Abstract

**Background: Objectives: Primary Objective:** To compare the ESR estimate by an automated analyser (ALI FAX Roller 20LC) and westergren method. **Secondary Objective:** To compare the ESR estimate by an automated analyser and westergren method in individuals with Tuberculosis, with Anaemia, in pediatric patients, and Male and female individuals with chronic inflammatory diseases. **Materials and Methods:** Blood is collected in black sodium citrate (3.8%) vacutainer under aseptic precautions. Then it is measured in westergren tube (mm per hour) and then same sample will be run in the Automated ESR analyser (ALI FAX Roller 20LC) in which reading is available in 30 mins. **Results:** The ESR measured by Westergren method ranged from 12mm/1st hour. to 127 mm/1st hour, with a mean of  $46.76 \pm 22.650$  mm/1st hour. while ESR measured by automated method ranged from 7.8 mm/1st hour to 128.7 mm/1st hr. with a mean of  $45.165 \pm 23.3753$  mm/1st hour. There was significantly strong correlation between automated and the Westergren method for determination of ESR ( $r = 0.933$ ;  $p = < 0.001$ ). Similar results were observed across all ages, gender, anemic and Tuberculosis patients. **Conclusion:** Automated and the Westergren method are strongly correlated. Automated method can be used in place of Westergren method for determination of ESR.

**Keywords:** Automated Method, Erythrocyte Sedimentation Rate, Westergren Method.

## Introduction

Erythrocyte sedimentation rate (ESR) is being used to evaluate the prognosis and diagnosis of certain diseases. It indicates the chronic inflammation that may be the pathological process in many ailments.[1]

Although it is non-specific, but this inexpensive test is commonly used in the diagnosis and monitoring of certain disabling conditions including Tuberculosis and Rheumatoid arthritis. This method is still considered as a reference standardized method .[2]

According to the recommendation of the International Council for Standardization in Hematology (ICSH) Westergren method is the method of choice for ESR measurement and subsequently introduced techniques for ESR measurement are being evaluated against this method. But, despite being a standard technique, it has certain disadvantages which limit its application. Therefore newer techniques of automated systems for measuring ESR have been introduced to overcome the disadvantages of the original technique.

In Westergren method the use of citrate, a liquid-based anticoagulant may affect the accuracy of ESR readings. The automated method uses EDTA as an anticoagulant, which is more reliable than the primarily used sodium citrate [3,4].

The automated ESR method helps in avoiding unnecessary exposure of blood samples and therefore reduces the risk to the sample handlers against the blood borne infections such as infectious hepatitis and HIV. This method also minimizes the possibility of external influence like temperature, dust particles, positioning of tube and diluents' ratio on the final reading. It also gives the ESR readings in much shorter time.

Studies have been conducted to compare the results of two methods but they show difference outcomes. Some concluded that there is agreement between two methods. While other show significant difference between the readings [5]. This study was aimed to determine the correlation between automated and the westergren method for determination of ESR in symptomatic patients.

## Methodology

This study was conducted at Alluri Sitarama Raju Academy of Medical Sciences, Eluru, West Godavari, Andhra Pradesh for a duration of around 3 months.

Total 500 blood samples from the patients referred at Hematology Department for evaluation of ESR and fulfilled the inclusion criteria, were selected by Non-Probability, Consecutive Sampling, included in the study. Time bound sample was taken as sample size.

Blood samples taking more than 30 seconds while collection or samples with excessive venous stasis samples without proper proportion to the anticoagulant were excluded from the study

Approval was taken from the ethical committee. 3ml blood sample was obtained by venipuncture into Tri potassium Ethylene diamine tetra -Acetic acid (K3-EDTA) vacuum tubes. The samples were thoroughly mixed at the time of venipuncture and also just before analysis.

Westergren method performed according to ICSH's specification was considered as standard/reference. Blood was transferred manually from an EDTA tube into ESR vacuum tubes containing sodium citrate. The sedimentation of the RBC's was recorded through visual determination, after 1 hour using a timer.

Automated ESR was performed by automated ESR analyzer (ALIFAX 20LC). This analyzer functions on the principle of photometrical capillary stopped flow kinetic analysis. It uses K3-EDTA or K2-EDTA as an anticoagulant.

Bias was controlled by inclusion and exclusion criteria and the confounding factors like temperature of lab, effect of wind were controlled by taking appropriate measures.

All the collected data was analyzed through SPSS version 26. Numerical variables; age, anemia, Tuberculosis patients whose ESR value by both the methods have been presented by mean  $\pm$ SD. Categorical variables i.e. gender has been presented by frequency and percentage. Pearson correlation coefficient analysis has been used to determine correlation between automated analyzer and Westergren method.

## Results

The mean age of the patients was  $38.63 \pm 19.730$  years. There were 252 (50.4%) male and 248 (49.6%) female patients in the study group. Among the causes for measurement of ESR: anemia is present in 128 patients (25.6%) and Tuberculosis patients are 56(11.2%) shown in Table 1.

**Table 1: Baseline Characteristics of Study Subjects**

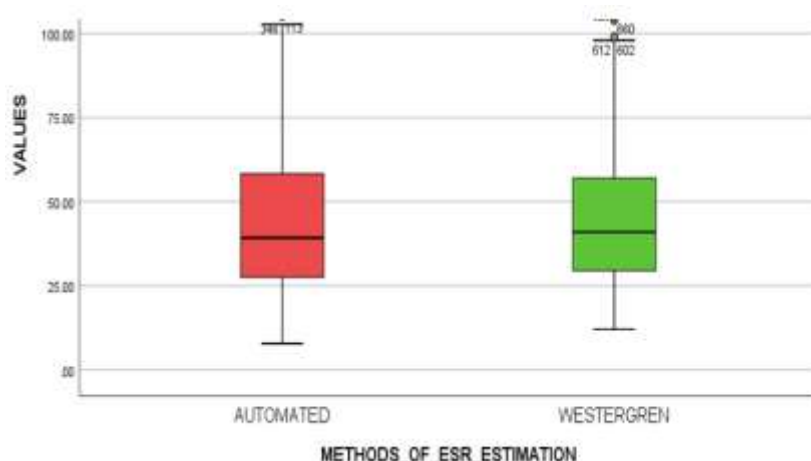
Variable		Count	Column N%
Gender	Male	252	50.4%
	Female	248	49.6%
Age category	1-14 years	48	9.6%
	15-49 years	314	62.8%
	50-84 years	138	27.6%
Anaemia	Present	128	25.6%
	Absent	372	74.4%
Tuberculosis	Present	56	11.2%
	Absent	444	88.8%

The ESR measured by Westergren method ranged from 12mm/1st hour to 127 mm/1st hour, with a mean of  $46.76 \pm 22.650$  mm/1st hour. While ESR measured by automated method ranged from 7.8 mm/1st hour to 128.7 mm/1st hr with a mean of  $45.165 \pm 23.3753$  mm/1st hour.

There was significantly strong correlation between automated and the Westergren method for determination of ESR ( $r = 0.933$ ;  $p = <0.001$ ). Similar results were observed across all ages, gender, anemic and Tuberculosis patients shown in Tables 2.

**Table 2: Correlation between Automated and Westergren ESR  $P < 0.001$  \*\* Correlation is significant at the 0.01 level (2-tailed).**

Variable		n	Automated	Westergren	Correlation
ESR (mean +/- SD)		500	45.15+/-23.37	46.76+/-22.65	0.933
Age	1-14 years	48	45.36+/-21.14	46.52+/-19.98	0.934
	15-49 years	314	45.14+/-25.45	46.62+/-22.07	0.955
	50-84 years	138	45.13+/-24.07	47.17+/-24.86	0.890
Gender	Male	252	45.10+/-23.26	46.40+/-22.38	0.960
	Female	248	47.13+/-22.95	47.13+/-22.95	0.906
Individuals with anemia		128	45.38+/-21.33	46.60+/-20.13	0.960
Individuals with normal Hb%		372	45.09+/-24.09	46.82+/-23.47	0.926
Individuals with Tuberculosis		56	45.21+/-24.81	45.36+/-23.21	0.962
Individuals without Tuberculosis		444	45.15+/-23.25	46.94+/-22.59	0.929

**Figure 1: Box and Whisker plot (Automated mean- 45.16, Westergren mean- 46.76. t value 1.097, p-value-.273)**

### Discussion

ESR is considered as a poorly understood test and despite the advent of newer techniques, no method certainly ruled out the effects of confounding factors like variations in relative erythrocyte volume or shape [6]. But, despite limitations, it remains a widely used test for the screening and monitoring of various conditions that affect plasma proteins and the sedimentation rate.

In developed part of the world, the traditional Westergren method is not commonly used in routine laboratories. But, it is considered as the gold standard technique for measuring ESR. Due to various disadvantages, its applications is now limited. To resolve this issue, several techniques to introduce automated systems for measuring ESR. Some of these involve automation of the Westergren method while others use very new technologies [7]. The

modification includes the use of undiluted EDTA samples, easier use, closed sample manipulation, conservation of time, vacuum controlled aspiration of the sample [8].

In this study significantly strong correlation between automated and the Westergren method for determination of ESR ( $r=0.945$ ;  $p= <0.001$ ) has been noted. The ESR measured by Westergren method ranged from 12mm/1st hour. to 127 mm/1st hour, with a mean of  $46.76 \pm 22.650$  mm/1st hour. while ESR measured by automated method ranged from 7.8 mm/1st hour to 128.7 mm/1st hr with a mean of  $45.165 \pm 23.3753$  mm/1st hour. There was significantly strong correlation between automated and the Westergren method for determination of ESR ( $r =0.933$ ;  $p = <0.001$ ). Similar results were observed across all ages, gender, anemic and Tuberculosis patients.

In the previously conducted studies, some authors found good correlation and agreement between two methods while few were noted significant discrepancies in the readings of the two methods. Asif *et al.* (2012) reported significant strong correlation between Westergren and automated methods ( $r=0.97$ ,  $p= 0.00$ ) similar to the present study [9].

Drashti in 2016[10], Kamal in 2018[10] and Hashemi *et al.* in 2014 ( $r=0.987$ ;  $p<0.001$ )[11], Sönmez *et al.* in 2014 ( $r=0.978$ ;  $p<0.05$ )[12], Cerutti *et al.* in 2011 ( $r=0.816$ ,  $p<0.05$ )[13] and Wiwanitkit in 2001 ( $r=0.98$ ;  $p<0.05$ ), also observed similar correlation between these two methods. Horsti *et al.* in 2010 reported relatively lesser correlation ( $r=0.72$ ;  $p<0.01$ ) between these two methods [14]. Venapusa[15], in his study determined that automated and Westergren method are correlated at 95%. But, simultaneously he noted higher ESR values with automated method.

Although, he associated his findings with the presence of systemic bias, but failed to determine its nature. Al fadhli[16], observed marked discrepancy in readings between the reference and the automated methods, but mostly with higher ESR readings; While, readings for normal and slightly raised ESR values were about similar by both methods. Similar variations were also noted by Plebani [17].

It can be thus advocated that automated method is also reliable and can be used for routine estimation of ESR. To determine more reliability on the automated analyzer, further validation experiments and studies, would be required.

## Conclusion

The results of the present study determined the significant correlation between the Westergren method and automated method of ESR estimation.

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