

A Comparative Study On Automated Esr Analyzer And Westergren Manual Method

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ABSTRACT

Introduction: Erythrocyte Sedimentation Rate (ESR) measures the rate of sedimentation of erythrocyte in millimeters of an anticoagulated whole blood. It is commonly used to screen for inflammation. This study aims to evaluate the performance of an automated ESR method in comparison with manual Westergren method and assesses its correlation across various demographic and clinical presentations.

Objective: To compare the utility of Automated ESR analyzer with routine Westergren Manual method.

Materials And Methods: A diagnostic study of 300 blood samples were analyzed for ESR estimation by Automated method and Westergren method. The study population was stratified by age, sex, and symptomatic status. Mean ESR values were compared across subgroups using appropriate statistical tests such as t-test. Pearson's correlation coefficient was used to assess the relationship between the two methods.

Results: A significant correlation was observed while comparing Automated and Manual Westergren method of Erythrocyte Sedimentation Rate with correlation coefficient of 0.987 ($r > 0.97$, $p < 0.001$) across all subgroups. Mean ESR values varied significantly with age and were slightly higher in females compared to males, consistent with known physiological variations. Symptomatic patients exhibited significantly higher mean ESR values compared to asymptomatic individuals.

Conclusion: This study demonstrates excellent correlation between the Automated method and Manual Westergren method across diverse demographic and clinical categories. The automated method offers a reliable, efficient, and potentially more precise alternative to the manual Westergren method for ESR determination. So, this study supports the replacement of the manual Westergren method with the automated method for routine ESR testing.

1. INTRODUCTION

The erythrocyte sedimentation rate (ESR) is a commonly employed hematological test used to detect inflammation and monitor disease activity. It measures the red blood cell settling in millimeters. ESR serves as a valuable indicator of systemic inflammation and is often used in conjunction with other clinical and laboratory findings. Edmund Biernacki developed the ESR test in 1897 (referred as Biernacki's reaction). However, it was Alf Vilhelm Albertson who defined the standardized methodology for ESR measurement which is currently considered the gold standard. Robert Ferguson noticed that ESR differs during pregnancy suggesting it could be used as a pregnancy indicator. The International Council for Standardization in Hematology (ICSH) recommends the Westergren method for ESR measurement¹. The automated method of ESR is recently used in most of the laboratories and the greatest advantage with this method is that it can give the ESR readings within minutes by temperature corrections at 18°C using infrared barriers which are not seen with the usual standardized methods for ESR². The rate of increase in ESR measurement needs an evaluation, because most of the inflammatory and neoplastic diseases are associated with it. ESR is a sensitive indicator of chronic inflammation even when asymptomatic, its measurement is often considered alongside a patient's clinical picture to provide a more comprehensive assessment³.

Erythrocyte Sedimentation rate is useful in (1) Any inflammatory or infectious or neoplastic etiology is suspected in the patients where specific diagnosis has not been evaluated. (2) The disease activity can be monitored in Tuberculosis, Rheumatic fever, Inflammatory arthritis, Giant cell arteritis, Polymyalgia rheumatica and Hodgkin disease. (3) It can be used

as a diagnostic criteria for Temporal arteritis and Polymyalgia rheumatica. When evaluating anemia in the presence of inflammation, the ESR can provide valuable information to differentiate between iron deficiency anemia and anemia associated with chronic disease. Correspondingly, the ESR may also serve as a useful indicator and predictor of lupus activity and organ damage respectively⁴.

2. MATERIALS AND METHODS

The diagnostic study was conducted at Clinical Pathology laboratory of Meenakshi Medical College Hospital and Research Institute, Kanchipuram for comparison of ESR by Automated and Manual method for a three-month duration from January to March 2024. Ethical committee approval was taken to conduct the study at our college. A total of 300 blood samples of symptomatic and asymptomatic patients were taken for analysis. The samples were collected according to inclusion and exclusion criteria and processed within 4 hours of collection. The inclusion criteria comprises of (1) Male and Female patients of age group from 20 to 75 years (2) Patients with various symptoms such as fever, malaria, cough, arthralgia and generalized weakness. (3) Asymptomatic Patients. The study excluded samples that were unsuitable for accurate ESR measurement. This includes (1) Improper blood to anticoagulant ratios (2) Lipemic and hemolyzed sample (3) Clotted blood as well as samples from patients with high bilirubin levels. The methods for estimation of Erythrocyte Sedimentation Rate (ESR) include Westergren method of ESR (Manual) and Automated method of ESR. Automated method which are commercially available includes SEDIMAT, Ves-Matic, ESR STAT - PLUS and Zeta sedimentation rate. These are adaptations of manual Westergren ESR method and they allow standardization of procedure which increases the accuracy of reproducibility. Automated Erythrocyte Sedimentation Rate testing offers several benefits like reduced labor costs, efficient use of blood samples, faster results and less risk of biohazard exposure for lab personnel. However, the initial investment in automated ESR equipment may be more expensive⁵. The International Council for Standardization in Hematology (ICSH) recommends Westergren method as a gold standard method because it uses Sodium citrate as anticoagulant which is liquid based and it may affect the accuracy of ESR readings. A single sample can be used for both hematological tests and estimation of Erythrocyte Sedimentation Rate. Accurate ESR measurement depends heavily on proper calibration due to variations in blood sample quality (anticoagulant type, collection tubes), measurement techniques and timing. Automated ESR methods offer an advantage by minimizing the impact of external factors like temperature, dust, tube position, and diluent ratios on test results. Additionally, automated systems offer great efficiency in sample processing compared to manual methods. This study aims to determine the correlation between the Erythrocyte Sedimentation Rate results obtained using the manual Westergren method and Peerless automated ESR analyzer. The requirements for manual method include Westergren ESR tube, a straight glass pipette measuring 30 mm in length and it is calibrated in mm from 0 to 200 (Top to bottom). The tube is open on both sides. The Westergren stand which holds the tube in a vertical position without any movement is required. The Venous blood was collected in 3.8% trisodium citrate solution in 4:1 (blood: citrate proportion). The sample was thoroughly mixed before analysis, kept at room temperature and run within four hours. The pipette was filled with K3 EDTA anticoagulated venous blood up to 200 mm mark and the erythrocyte sediment was noted at 60 minutes in mm/hour. In automated method for estimation of red blood cell sedimentation rate, Peerless automated analyzer was used. This automated analyzer has a special vacutainer so that blood can be directly drawn into it. The blood should be filled up to the marked level and mix the blood sample before putting into automated analyzer. The automated analyzer works on the principal of infrared led detection. This autoanalyzer will give the results of ten samples in thirty minutes which is equal to one hour. All the samples were processed and then collected data was recorded. Through the SPSS latest version, the data was analyzed. Mean \pm Standard deviation of automated and manual ESR values was calculated according to age, gender symptoms which include fever, malaise, cough, arthralgia, generalized weakness and asymptomatic patients. Pearson correlation coefficient analysis was used for the determination of correlation between manual and automated method of ESR.

3. RESULTS

Statistical analysis was done using SPSS software latest version. Descriptive statistics were used to find the frequency percentage of age, gender, symptomatic and asymptomatic patients. Data was normally distributed and so the correlation between the automated and manual ESR measurements were assessed using Pearson correlation coefficient.

The demographic characteristics of the study population (n=300) are presented in Table 1 demonstrating a balanced representation of 153 male and 147 female patients.

The patients were categorized into age group of intervals from 20 to 25 years, 25 to 50 years and 51 to 75 years. There were 140 patients in 25 to 50 age group, 114 patients in 51 to 75 age group and 46 patients in 20 to 25 age group. Distribution among age group is shown in Table 2.

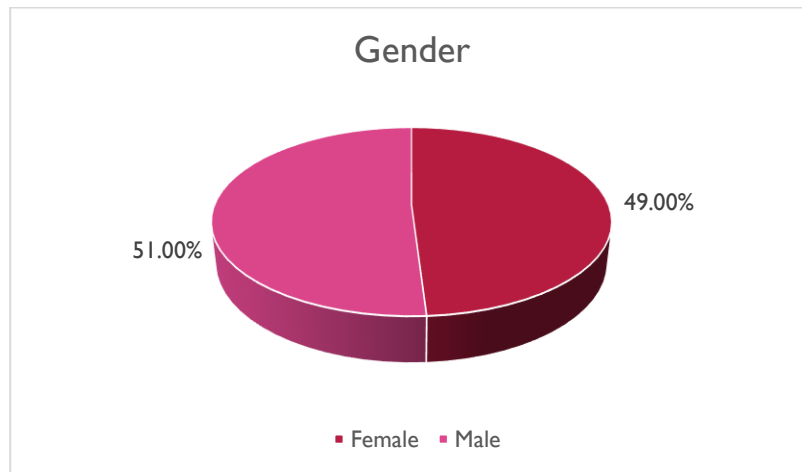
The patients were categorized according to symptom presentation (symptomatic or asymptomatic). The distribution of patients within these classifications is shown in Table 3.

Symptomatic patients were categorized according to fever, cough, generalized weakness, arthralgia and malaise. There were 44 patients with fever, 36 patients with cough, 26 patients with generalized weakness, 20 patients with the arthralgia and 16 patients of malaise. The distribution of patients according to symptoms is shown in Table 4.

TABLE 1:

Gender	Frequency n(%) n=300
Male	153(51.0%)
Female	147 (49.0%)

Table 1 shows the distribution of patients based on gender with a balanced representation of male (51%) and female (49%) participants.

Figure 1 Pie chart based on the gender distribution of the patients**TABLE 2:**

Age group (45.44±16.785)	
20-25	46(15.3%)
25-50	140(46.7%)
51-75	114(38.0%)

Table 2 shows the distribution across age group. There were 46.7% in 25 to 50 years, 38% in 51 to 75 years and 15.3% in 20 to 25 years of age group.

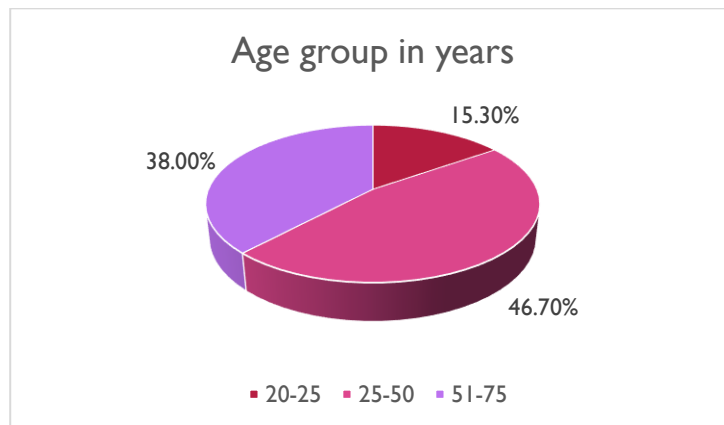
Figure 2 Pie chart based on the age distribution of the study patients

TABLE 3:

Symptomatic Patients	142(47.3%)
Asymptomatic Patients	158(52.7%)

Table 3 shows the distribution across symptomatic and asymptomatic patients. 47.3% patients were in the symptomatic category while 52.7% were asymptomatic

TABLE 4:

	Frequency n(%) n=142
Symptomatic Patients	
Fever	44(30.98%)
Cough	36(25.35%)
Generalized Weakness	26(18.30%)
Arthralgia	20(14.08%)
Malaise	16(11.26%)

Table 4 shows the distribution across symptomatic patients with fever, cough generalized weakness, arthralgia and malaise. There were 30.98% of fever cases, 25.35% of cough cases, 18.30% of generalized weakness cases, 14.08% of arthralgia cases and 11.26% of malaise cases.

Mean \pm standard deviation was calculated. The mean age of the patient was 45.44 ± 16.785 years. The mean of the automated ESR method was 28.08 ± 22.016 and Manual method was 27.86 ± 21.533 . Mean \pm standard deviation and Pearson correlation coefficient analysis were shown in Table 5 to Table 8 and figure 1 to figure 5. There was a significant strong correlation between the automated and manual method for detection of ESR with ($r=0.987$, $p<0.001$) which signifies that there is a positive correlation.

Table 5: Correlation between Automated and Manual ESR among the Age groups.

		N	Automated	Manual	Correlation
ESR (Mean\pmSD)		300	28.08 ± 22.016	27.86 ± 21.533	0.987**
Age	20-25	46	19.59 ± 17.787	19.59 ± 17.833	0.973**
	25-50	140	25.16 ± 18.349	25.26 ± 17.739	0.984**
	51-75	114	35.09 ± 25.552	34.40 ± 25.174	0.990**

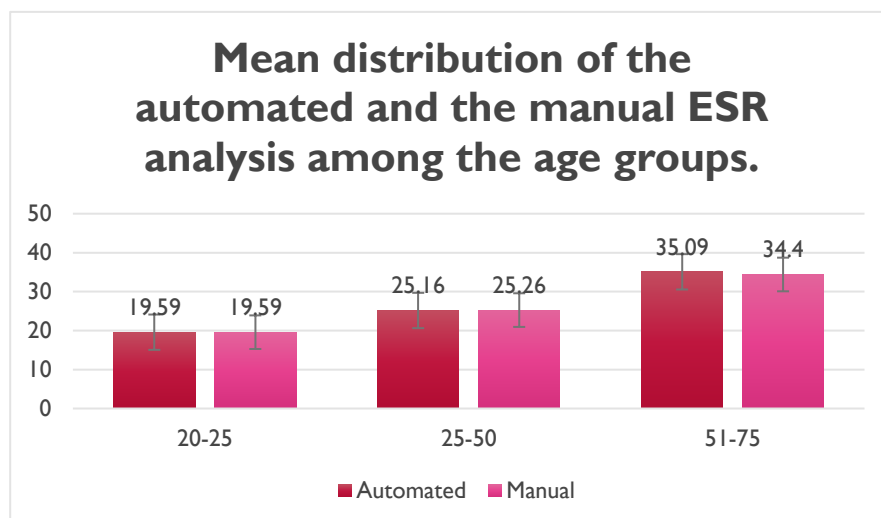
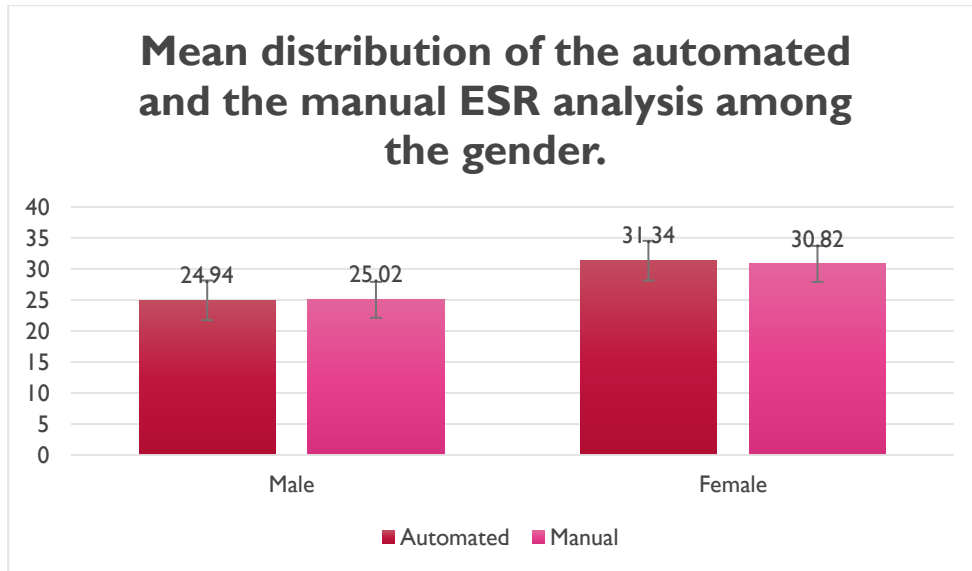
**Figure 3 Bar chart with mean distribution of the automated and the manual ESR analysis among the age groups.**

Table 6: Correlation between the Automated and the Manual ESR among the Gender.

Gender	Male	153	24.94±22.725	25.02±22.121	0.990**
	Female	147	31.34±20.832	30.82±20.564	0.982**

Figure 4 Bar chart with mean distribution of the automated and the manual ESR analysis among the gender**Table 7: Correlation between Automated and Manual ESR among the Symptomatic and Asymptomatic Patients**

Category		N	Automated	Manual	Correlation
Symptomatic patients		142	45.27±20.460	44.41±20.503	0.979**
Asymptomatic patients		158	12.63±6.297	12.99±5.951	0.897**

Table 8: Correlation between Automated and Manual ESR among various Symptoms

Symptoms	N	Automated	Manual	Correlation
Fever	44	46.47±21.849	46.27±21.952	0.975**
Cough	36	54.837±21.204	53.22±20.411	0.983**
Generalized weakness	26	32.11±11.683	31.07±11.603	0.958**
Arthralgia	20	51±20.238	50±22.030	0.975**
Malaise	16	33.18±8.134	34.62±9.076	0.932**

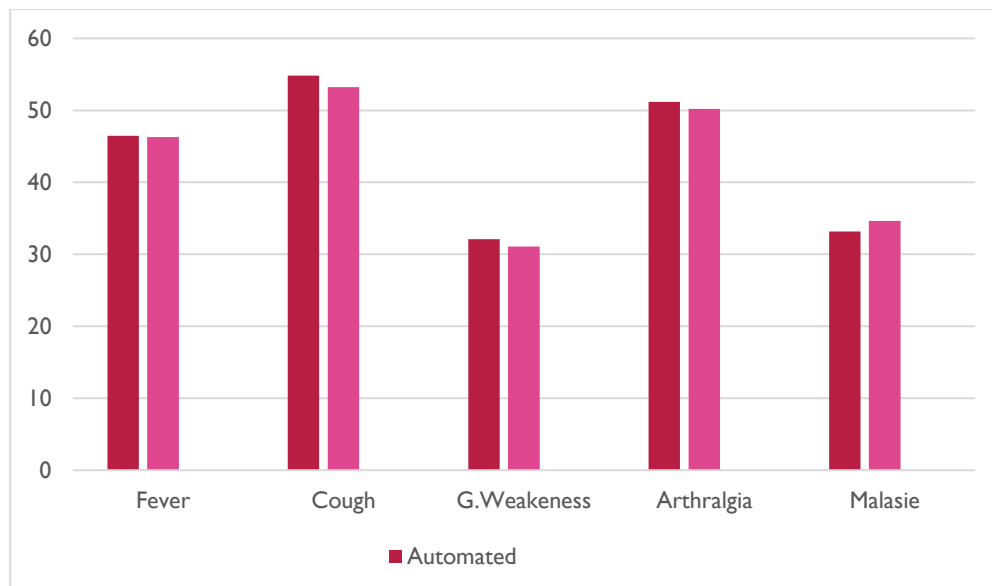


Figure 5 Mean distribution among various symptomatic patients

4. DISCUSSION

The Erythrocyte Sedimentation Rate is a laboratory test that determines the rate at which red blood cells descend to the bottom of a vertical tube within a specified timeframe. The principle of the ESR test involves observing the settling of red blood cells in a vertical column of anticoagulated blood over a one - hour period. The result is expressed in millimeter. The negative charge on red blood cells causes them to repel each other, but this repulsion can be diminished or neutralized by increased levels of positively charged plasma proteins. Increased protein in plasma reduces negative charge on the surface of red cells and reduces the electrical repulsion among red blood cells and this red blood cells gets closer together and facilitates Rouleaux formation. Erythrocytes sedimentation typically proceeds in three stages. The first stage includes the Rouleaux formation. During the initial period of 10 minutes a little sedimentation is formed as the red cells stack together like a pack of coins. The size of Rouleaux formed directly affects the sedimentation rate which is the most critical stage in determining the ESR. Stage two includes the sinking of Rouleaux formation in 40 minutes. There is a rapid and constant sedimentation. The longer is this tube, the higher is the value of ESR. Both packing of Rouleaux and slow sedimentation take place during the last 10 minutes of stage 3. After an hour, the rate of sedimentation of erythrocyte is measured.

The normal range of erythrocyte sedimentation rate according to age and gender is summarized below:

Male under 50 years: 0 to 15mm in 1hour

Male over 50 years: 0 to 20mm in 1 hour

Female under 50 years: 0 to 20mm in 1 hour

Female over 50 years: 0 to 30mm in 1 hour

Newborn: 0 to 2mm in 1 hour

Children to Puberty: 0 to 13mm in 1 hour

ESR naturally increases with age even in absence of disease. Woman tends to have slightly higher ESR values, especially during menstruation and pregnancy. Excess weight and drugs like oral contraceptives and statins may increase ESR. An elevated ESR in an asymptomatic individual is not always a cause of concern but should not be ignored, especially if levels are manually high or persistent. It is essential to monitor the trends and perform additional tests to rule out any hidden conditions. In many cases, ESR elevation resolves without intervention but with regular follow up. Routine follow up and correlation with clinical findings are essential to ensure no significant underlying issue is missed. The ESR is affected by both erythrocyte related factors such as erythrocyte characteristics (size and count) and plasma components (fibrinogen and immunoglobulins)⁶.

The causes of increased ESR in general are divided into several categories such as infectious causes, inflammatory causes, acute myocardial infarction, malignancy and paraproteinemia's. Infectious causes include bacterial, viral, fungal, parasitic infections, acute rheumatic fever, osteomyelitis, tuberculosis and acute hepatitis. Inflammatory causes include Rheumatoid arthritis, Systemic lupus erythematosus, Temporal arteritis, Polymyalgia rheumatica and Kawasaki disease. Extreme elevation of ESR occurs in coronary artery involvement. In emergency situation, ESR may be helpful in distinguishing angina pectoris

from myocardial infarction. Anemia and polycythemia represent changes in erythrocyte quantity, observed in various clinical conditions which will cause the ESR to either rise or fall⁷. Technical factors also show a falsely elevated ESR. The ESR tube should be kept vertical and there should be no vibration. If there are air bubbles inside the tube or the room temperature is low or the time is too short, erythrocyte sedimentation rate will be reduced.

In our study, the comparison of ESR by Manual Westergren method and Automated method across different sub groups showed a significant correlation ($r=0.987$, $p<0.001$) among age, gender, symptomatic and asymptomatic patients. The distribution and correlation across different subgroups are discussed below.

Distribution and Correlation among Gender

Table 1 reveals a near-equal representation of both sexes, with 153 (51%) males and 147 (49%) females. The even representation of males and females helps to minimize potential gender-related bias in the subsequent analysis of ESR values. Since ESR can be influenced by physiological differences between sexes (e.g., hormonal variations, red blood cell characteristics), an imbalanced sample could skew the overall results and make it difficult to generalize findings to the broader population. The correlation between automated and manual Westergren ESR measurements, stratified by gender shown in Table 6 shows a strong positive correlation between the two methods in both male and female patients, with some notable observations regarding mean ESR values. The high correlation coefficients observed in both male and female groups (0.990 and 0.982, respectively; $p < 0.01$ for both). The strong correlation between automated and manual ESR across both genders suggests that the automated method can be reliably used in both male and female patients. This eliminates gender as a potential confounding factor when comparing results obtained by different methodologies.

Gender-Related Differences in Mean ESR

The data reveals a statistically significant difference in mean ESR values between male and female, while females exhibiting higher mean ESRs compared to males. This finding is consistent with established physiological norms and reflects known gender-related variations in ESR. These differences are primarily attributed to hormonal influences on factors affecting ESR, such as red blood cell size and plasma protein composition. It is crucial to acknowledge these physiological gender differences when interpreting ESR results in clinical practice.

Distribution Across Age Groups

The largest proportion of patients (46.7%) falls within the 25–50 year age group, followed by the 51–75 year group (38%), and the smallest group is the 20–25 year group (15.3%). Age is a known factor that can influence ESR values. As individuals age increase, there is a tendency for ESR to increase even in the absence of underlying disease. Therefore, it is essential to consider the age distribution of the study population when evaluating and interpreting ESR results, especially when comparing different measurement methods. The mean age of 45.44 years indicates that the study population is primarily composed of middle-aged adults. The overall correlation coefficient of 0.987 ($p < 0.001$) indicates an exceptionally strong linear relationship, suggesting that the automated method closely mirrors the performance of the manual Westergren method across the entire age range studied. This is further reinforced by the strong correlations observed within each age subgroup: 0.973 for 20–25 years, 0.984 for 25–50 years, and 0.990 for 51–75 years (all $p < 0.001$).

Age-Related Variation in Mean ESR

Mean ESR values show a tendency to increase with age. This observation aligns with established physiological norms and reflects age-related changes in factors influencing ESR, such as alterations in plasma protein composition and red blood cell properties. The observed increase underscores the importance of considering age as a potential variable when interpreting ESR results. However, importantly, despite the variation in absolute ESR values across age groups, the strong correlation between the automated and manual methods remains consistent, demonstrating the reliability of the automated method across the physiological spectrum of ESR values associated with aging.

Distribution and correlation across symptomatic and asymptomatic patients

The distribution of 300 patients included in this study based on their symptomatic or asymptomatic status. The table 3 reveals that 142 patients (47.3%) were classified as symptomatic, while 158 patients (52.7%) were classified as asymptomatic. The division of patients into symptomatic and asymptomatic groups is clinically relevant as ESR values can be influenced by the presence of underlying inflammatory processes or infections, which are more likely to manifest with symptoms. Comparing ESR measurements between these two groups can shed light on the utility of ESR in different clinical contexts. While an elevated ESR in a symptomatic patient may readily suggest inflammation, interpreting ESR values in asymptomatic individuals requires careful consideration of potential subclinical conditions or other factors that could influence ESR. Among symptomatic patients, Fever and cough were the most frequently reported symptoms, followed by generalized weakness, arthralgia, and malaise. The specific symptoms reported provide valuable information about the potential underlying causes of the elevated ESR. For instance, the presence of fever and cough might suggest respiratory infections, while arthralgia could point towards inflammatory joint conditions. the most common diagnoses associated with these symptoms in your study population. The table 7 demonstrates a positive correlation coefficient of 0.979 ($p < 0.01$) for

symptomatic patients and 0.897 ($p < 0.01$) for asymptomatic patients regardless of the presence of symptoms between the automated and manual ESR methods.

The correlation between automated and manual Westergren ESR measurements in Table 8 is categorized by specific symptoms present in patients. The data reveal a strong positive correlation between automated and manual ESR measurements across all reported symptoms, including fever, cough, generalized weakness, arthralgia, and malaise. The correlation coefficients range from 0.932 to 0.983 ($p < 0.01$ for all), indicating a strong correlation between the two methods regardless of the specific symptom. This suggests that the automated method provides comparable results to the manual Westergren method in patients presenting with a variety of clinical complaints.

Variation in Mean ESR by Symptom

The mean ESR values are significantly higher in the symptomatic patient group compared to the asymptomatic group. This difference is consistent with the understanding that ESR is often elevated in the presence of inflammatory processes, which are more likely to manifest with symptoms. The observed difference underscores the clinical utility of ESR as an indicator of inflammatory activity. While the correlation between the methods is consistently strong, the mean ESR values do vary across different symptom categories. For instance, patients presenting with cough tend to have higher mean ESR values compared to those with generalized weakness or malaise. This variation likely reflects the diverse underlying pathophysiological processes associated with these symptoms. Cough, for example, may be indicative of respiratory infections or inflammatory conditions, which are often associated with elevated ESR. Arthralgia may suggest inflammatory joint conditions. ESR is a useful indicator of inflammation, its non-specificity necessitates careful consideration of the clinical picture, including specific symptoms, to arrive at an accurate diagnosis. However, further research focusing on specific symptom-based stratification is needed.

Studies have proved that some authors found a strong correlation and some found discrepancy between automated and manual method of erythrocyte sedimentation rate. Similar to this study, Itty compared the ESR values between three methods (Manual modified Westergren method, automated Vesmatic Cube 30 and automated Mixrate X20). Here they used two automated systems to compare with Westergren method. The comparison of the ESR values across age and gender showed a significant correlation (manual vs Mixrate x 20, $r = 0.891$ and manual vs Vesmatic Cube 30, $r = 0.998$)⁶. Hina Mushtaq, stated that there was a significantly strong correlation between automated and the Westergren method for determination of ESR ($r = 0.945$) across all ages, gender, duration and type of symptoms groups¹.

Asif, compared the ESR values of 108 blood samples by Ves-Matic Easy and Vacuette SRS 20/11 with Westergren method and found a strong positive correlation ($r = 0.97$)⁸. Cerutti, compared the ESR of 248 patient's samples by Westergren and VES Matic methods and found a good correlation $r = 0.816$ ⁹. Drashti, compared the ESR of 500 patients. The patient were subdivided into 6 groups on the basis of clinical diagnosis and their study revealed a strong positive correlation among the subgroups and he also noted discrepancy among the orthopedic patients¹⁰. Wiwanikit, compared the ESR by two methods with 80 individual volunteers and found a good correlation ($r = 0.945$)¹¹. Similar findings noted by Fiorucci et al (2000) reported significant strong correlation between westergren and automated methods $r = 0.99$ ¹² and Pirovcic et al (2010) $r = 0.95$ ¹³.

Some discrepancies noted by authors include, Venapusa (2017) found a 95% correlation between automated and Westergren ESR methods, but observed generally higher values with the automated method¹⁴. Al Fadhli (2018) reported significant discrepancies, primarily with higher automated ESR readings, though normal and slightly elevated values were similar between the two methods¹⁵. Plebani (2001) also noted similar discrepancies¹⁶.

Hence, further experiments are needed for the comparison between two methods to ensure the measurements are accurate since only few studies are done especially across different clinical presentations.

5. CONCLUSION

This study, comparing automated and manual (Westergren) methods for erythrocyte sedimentation rate (ESR) determination, reveals a strong positive correlation between the two. The good correlation observed across various patient subgroups (defined by age, gender, and symptomatic status) indicates that the automated method can provide accurate results. Furthermore, the automated method offers advantages in terms of efficiency, reduced hands-on time for laboratory personnel, and potentially decreased turnaround time. Therefore, based on these findings, the automated ESR method can be confidently adopted as a reliable and efficient alternative to the manual Westergren method in clinical practice. Further studies can be done to evaluate the cost-effectiveness and long-term performance of the automated method in diverse clinical settings.

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