Original Research Article

Correlating Automated IRIA ESR Analyzer and Westergreen Method for Determination of Erythrocyte Sedimentation Rate

Abstract: The erythrocyte sedimentation rate (ESR), or sedimentation rate (sed rate), is a measure of the settling of red blood cells in a tube of blood during one hour. The rate is an indication of inflammation and increases in many diseases. Westergreen method is routinely used determination of ESR, however, it requires large volume of blood and it is time consuming as it takes one hour for analysis. In order to overcome these challenges, new technologies have implemented an automated system which saves on labour, no need for aliquots, shorter turnaround time and minimizes exposures of laboratory staff to biohazard risks. The main objective of this study was to compare Westergreen method and automated IRIA analyzer in determination of ESR. The specific objectives were to determine the ESR using both Westergreen tube method and automated IRIA analyzer and to determine the correlation between Westergreen tube method and automated IRIA analyzer methods. This cross-sectional study was carried out in 205 blood samples at Polyclinique La Medicale from August 2017 to April 2018. Data were analyzed using SPSS, version 21. The current study included a total of 205 participants 123 (60%) females and 82 (40%) males. For a total of 205 participants, the normal tests on westergren method were 142 (69.3%) and abnormal tests were 63 (30.7%), whereas for automated IRIA ESR analyzer, 131 (63.9%) were normal and 74 (36.1%) tested abnormal. There was a strong correlation between automated IRIA ESR analyzer and Westergreen method with r=0.9. The authors recommend that IRIA ESR analyzer should be used in determining ESR due to its advantages over Westergreen method.

Keywords: Correlation, Westergreen Method, Automated IRIA ESR Analyzer, ESR

1. Introduction

Erythrocyte sedimentation rate (ESR) Erythrocyte sedimentation rate (ESR) is an hematological parameter mostly used to investigate different diseases and disorders. It is still the most reliable test in most clinical facilities although it has long turnaround time and requires too much blood [12]. In 1977, new documents were published by the International Council for Standardization in Hematology (ICSH) and the National Committee for Clinical Laboratory Standardization (NCCLS). Acceptable modifications to the routine method were stated, such as pipettes made of plastic rather than glass, as well as the use of EDTA-anticoagulated blood [1]. In 1988, both NCCLS and ICSH published new guidelines for quality assurance. In 1993, an ICSH group published new recommendations, stressing the importance of ensuring that measurements obtained in different laboratories were comparable [1]. Several new methods, some of them automated or semi-automated, became available in 2001. The technical innovations incorporated in these new instruments significantly improved on the existing procedures. Some of the new methods had shorter testing times, others had reduced the biohazards of ESR testing as the samples were aspirated from closed tubes, avoiding exposure of personnel to blood. The CLSI H02-A4 standard covered the new instruments that were available at the time [8]. Despite these efforts, the international standardization and comparability of ESR methods remained unsatisfactory. ICSH and CLSI therefore made new recommendations in 2010 and 2011. The ICSH document recognized that automated methods were routinely used in many laboratories, using diluted or undiluted samples, but the reference procedure remained Westergreen method. The document stated that all new technologies, instruments, or methodologies had to be evaluated against the Westergreen reference method before being introduced into clinical use and that “systems that give the results as the Westergreen method with diluted blood at 60 minutes or normalized to 60 minutes are the only ones of clinical value (ICSH 2011). It was recommended that manufacturers provide data on the reliability and trueness of any method and instrument, as well as calibration and control procedures. A protocol for evaluation of the routine/working method against the standardized method was also described, clearly indicating the statistical methods that should be used for the comparative evaluation [2].

At present, standardization in this field is facing

automation and novel methods to measure the ESR. These pressures are inevitable because of increased workloads, cuts in laboratory personnel and budgets, and the need for closed blood collection tubes to ensure employee safety. The new technologies and instruments address many of these concerns and are therefore attractive to many laboratories. Because of these changes, there is a need for a continuing improvement in the harmonization of the ESR [13, 14].

It is evident that till now, there are still significant variations in the methodology used to determine ESR. The National Committee for Clinical Laboratory Standards (NCCLS; now called Clinical Laboratory Standards Institute [CLSI]) and the International Council for Standardization in Hematology (ICSH) published methods for standardizing performance of the ESR and the Westergreen method was selected as the reference method as it was reliable, reproducible, and sensitive. The defined standardized method recommended the use of blood diluted with trisodium citrate dehydrate and specified the technique, including dimensions and characteristics of the pipettes and how to report the results, namely as millimeter sedimentation after 60 minutes [5]. Working Group consisting of the six authors of this study was convened by the ICSH. Advantages of integration of ESR technology into automated systems include savings on labor, no need for aliquots and therefore more efficient use of sample volumes, shorter turnaround times, and minimal exposure of laboratory staff to biohazards. Disadvantages include possible higher costs of instrumentation.

There are several different methods to determine the ESR, but the conventional Westergreen method is still referred to as the reference method for measurement of ESR and for validation of new ESR methods. This method determines erythrocyte sedimentation after 1 hour in a vertically mounted tube of defined length and bore size, thereby analyzing all 3 phases in the process of erythrocyte sedimentation: aggregation, sedimentation, and packing [3]. For practical reasons, the Westergreen method itself is sparsely used in the routine determination of the ESR. It carries a risk of infection (open tubes), needs relatively large volumes of blood, and, with an analysis time of 1 hour, hence it is time-consuming. To overcome the practical drawbacks of the conventional Westergreen ESR method, several methods based on the conventional Westergreen method were introduced [15]. According to international council of standardization in hematology, westergreen tube method slows turn around time, it is conceived in a system of open tube which exposes to biohazard risk. The determination of the ESR by the westergreen tubes method is affected by the diameter of the tube and ICSH specifies an international diameter of 24-27mm [2]. On the other hand, Westergreen tube method requires dilution of four parts of blood to one part of citrated diluents and this dilution steps is a potential cause of poor quality control: as the ratio of blood to citrate increases, the dilution effect of the anticoagulanton the plasma concentration of rouleau- inducing proteins is decreased so that more rouleau form and the ESR increases [4, 7].

The advantages of integration of ESR technology into automated systems include savings on labor, no need for aliquots and therefore more efficient use of sample volumes, shorter turnaround times, and minimal exposure of laboratory staff to biohazards, however its disadvantages include possible higher costs of instrumentation. According to New ICSH recommendations for modified and alternate ESR methods Laboratories that want to introduce modified and alternate ESR methods are obliged to follow all applicable regulatory and institutional requirements [13].

Laboratories must confirm the instrument's accuracy by comparing results to their predicate method. At least 30 samples spanning the analytical range of the instrument should be compared [4]. Although, westergreen tube technique has many disadvantages, in Rwanda, there is no other advanced method that was developed or validated for overcoming these disadvantages. Hence, this study was of great importance to be conducted. The general objective of this study was to correlate automated IRIA analyzer and Westergreen method for determination of erythrocyte sedimentation rate.

2. Materials and Methods

The current study was conducted at POLYCLINIQUE LA MEDICALE located near Centre Saint PAUL, at Nyarugenge district in Kigali City, Rwanda. A cross-sectional study was carried out on patients who attended Polyclinique LAMEDICALE during the period from August 2017 to April 2018. Citrated (Seditainer ESR tubes, Becton Dickinson) or EDTA-anticoagulated (in Vacutainer tubes, Becton Dickinson) whole blood were used to determine the ESR within 4 hours after blood collection. ESR from 205 citrated blood samples was determined using both Westergreen method and automated IRIA ESR analyzer. Samples were obtained using convenience sampling technique. During ESR determination, the ESR tubes were placed on the Westergreen rack and the red blood cells were allowed to settle for an hour, after which the ESR was measured in mm/hour. At the same time, the IRIA well of an analyzer was filled with blood sample and analyzed automatically in the automated IRAIA analyzer. The results of ESR were determined in mm/hr. Results were kept using file records as well as electronically for better management of the data. The results obtained from both ESR testing methods were compared and further analysis was made. In addition, Westergreen method served as the gold standard reference method according to approved standards of hematology regarding ESR determination in clinical setting. The correlation between ESR results obtained from two different testing approaches was determined by applying Karl Pearson’s formula.

Ethical Consideration

This study has been revised and approved by a departmental Institutional Review Board committee within the school of Health Sciences of Mount Kenya University, Kigali. Ethical approval has been requested from research committee Polyclinic La Medicale. To assure confidentiality, the new Numbers were used as study ID instead of names or hospital ID on patient data extraction forms.

3. Results

3.1. Demographic Characteristics of Study

Subject

Table 1 shows that, among all participants 123 (60%) were females and 82 (40%) were males.

Table 1. *Demographic characteristics of the study participants.*

Age	Gender		Total
	Females	Males	
16-24	22	13	35 (7%)
25-34	31	12	51 (24.8%)
35-44	22	20	38 (18.5%)
45-54	30	20	42 (19%)
55+	18	17	39 (19%)
Total	123 (60%)	82 (40%)	205 (100%)

The majority being between 45 and 54 years old and the minority being above 55 years old.

3.2. Abnormal and normal results from both automated IRIA ESR analyazer and Westergreen methods

Table 2. *ESR results from both Westergreen method and automated IRIA ESR analyzer.*

TEST METHOD	Normal %	Abnormal %	Total %
WESTERNGREN %	142 (69.3%)	63 (30.7%)	205 (100%)
IRIA analyzer %	131 (63.9%)	74 (36.1%)	205 (100%)

Table 2 shows the results got from both IRIA analyzer and Westergreen method. Among 205 patients tested for Erythrocyte Sedimentation Rate, using IRIA analyzer, 74 (36.1%) were having elevated ESR (abnormal values indicating possible inflammatory diseases, ESR>20mm/hour) whereas 131 people were having normal values. On the other hand Westergreen method, abnormal results were 63 (30.7%) and 142 (69.3%) of people were having normal values.

Mean average of ESR values using Westergreen method and automated IRIA analyzer

3.3. Mean results of ESR using Westergreen method and automated IRIA ESR analyzer.

Table 3. *Mean average of ESR using Westergreen method and automated IRIA analyzer.*

METHODS	N	ESR VALUES			
		Minimum	Maximum	Mean	SD
ESR IRIA ANALYZER (in mm/hr)	205	1	113	24.09	24.46
WESTERGREEN METHOD (in mm/hr)	205	1	114	23.12	25.91

Table 3 illustrate the mean average, minimum, maximum and standard deviation of the results of ESR measured in mm/hour. The table shows that among 205 tested samples, the minimum ESR value was 1mm/hour for both ESR IRIA analyzer and Westergreen method. The maximum ESR measured using westergreen method was 114mm/hour whereas it was 113mm/hr for IRIA analyzer. The mean average of ESR for Westergreen method and IRIA analyzer were 23.12 and 24.09 mm/hour respectively. The SD of ESR results were 24.46 and 25.91 for ESR IRIA analyzer and Westergreen method respectively. There were no significant difference between means using ESR analyzer and Westergreen method (P=0.1).

3.4. Determination of the Correlation Between Westergreen Method and Automated IRIA ESR Analyzer

Karl Pearson'sCoefficient of Correlation

Procedure for computing the correlation coefficient

Various values has been calculated by substituting the values in the formula:

Sfdxdy, Sfdx, Sfdx2, Sfdy, Sfdy2 to get the value of r(Young et al., 1999).

$r = N \Sigma dxdy - \Sigma dx \Sigma dy / \sqrt{N \Sigma dx^2 - (\Sigma x)^2} \sqrt{N \Sigma dy^2 - (\Sigma dy)^2}$ (Young et al., 1999).

dX= 20.055 dY=15.08

dxdy=115592.9

$dX^2 = 137000.7$

$dY^2 = 122055.2$ x=4721y=49N=205

r=N(20.055x15.08)-(20.055x15.08) /√N(137000.7-(4721)²√N(122055.2-(15.08)²

r=205(20.055x15.08)-(20.055x15.08)/ √205(137000.7-(4721)²√205(122055-(15.08)²=

r= 0.8939= 0.9

Interpretation of Correlation Coefficient (r)

The value of correlation coefficient 'r' ranges from -1 to +1

If r = +1, then the correlation between the two variables is said to be perfect and positive

If r = -1, then the correlation between the two variables is said to be perfect and negative ,,

If r = 0, then there exists no correlation between the variables

value strength of correlation

r2 = 0 no correlation, 0 < r2 < 0:25 very weak correlation, 0:25 6 r2 < 0:50 weak correlation

0:50 6 r2 < 0:75 moderate correlation, 0:75 6 r2 < 0:90 strong correlation

0:90 6 r2 < 1 very strong correlation

r2 = 1 perfect correlation

4. Discussion

The main objective of this study was to correlate the Westergreen method and automated IRIA analyzer for determination of erythrocyte sedimentation rate. There was a strong correlation between Westergreen method and automated IRIA analyzer as the r=0.9. Actually, the Correlation is a statistical measure that indicates the extent to which two or more variables fluctuate together. A positive correlation indicates the extent to which those variables increase or decrease in parallel; a negative correlation indicates the extent to which one variable increases as the other decrease. In this case the correlation between Westergreen and automated IRIA analyzer is positive which indicates that the values of Westrgreen may increase or decrease in parallel with that of automated IRIA analyzer which implies a strong correlation.

The conventional manual Westergren method is still considered the reference method for the measurement ofESR, despite its intrinsic practical drawbacks such as risk of infection and relatively long analysis time. The introduction of Westergreen-based semi-automated methods has substantially improved the application of ESR measurement. In line with the Westergreen reference method, these automated methods dilute whole blood with citrate, measure sedimentation of erythrocytes in dedicated tubes, and, subsequently, recalculate to conventional Westergreen units.

Automated methods generate fast and reliable ESR measurements and show good correlation with the conventional Westergreen reference method. More recently developed ESR methods circumvent the need for additional dilution and thereby optimize logistical laboratory workflow, enhance operator safety, and reduce laboratory waste. The IRIA ESR analyzer is an example of such a modern automated ESR method that uses standard citrated blood sample tubes for direct measurement of erythrocyte sedimentation.

In a study that was conducted in by Sezer et al., 2013 [10]. Ves Matic Cube 200 instrument was compared with Bland-Altman analysis method using Westergren method in which the correlation coefficient was 0.82. The correlation (r) of ESR results from the SEDIsystem and the StaRRsed system with Westergren method was 0.96 [10]. This agrees with the findings from the current study where the correlation coefficient was found to be 0.9

In a study done by Öztürk *et al.,* 2014, 2methods namely iSed Alcor Auto-instrument and Berkhun SDM60 Auto-instrument were compared based on their consistence for measurement of Erythrocyte Sedimentation rate. The findings from this study showed a strong correlation between the two automated methods with r equal to 0.90, however the findings were not compared with westergreen methods which would have revealed the use of these aumated essays over westergreen method, a gold statndard method for measuring ESR [11].

Hashemi *et al.,* 2015 conducted a study on Erythrocyte Sedimentation Rate Measurement Using as a Rapid Alternative to the Westergreen Method. The study compared automated Micro ESR method against Westergreen method using Pearson and Spearman's coefficients. The findings showed a strong correlation between these two method with n r== 0.987 and r^2=0.974 [12].

A research conducted by [15], computerized tube viscometer method was compared with conventional westergreen method in determination of ESR where the correlation between the two methods was 0.92. The results were generated in 4minutes using computerized tube viscometer method compared to Westergreen method which generates ESR results within one hour.

5. Conclusion

The aim of this study was to compare analytic performances of the IRIA analyzer and Westergren-based citrated methods. From the findings of the current research project, Erythrocyte Sedimentation rates measured with both methods were compared and they revealed a strong positive correlation with r equal to 0.9. In summary, these findings indicate that IRIA ESR analyzer is reliable and suitable system for high workload clinical laboratory. It will be vital to carry out further studies in order to determine the correlation of the two methods in cases of blood disorders including Polycythaemia, Poikilocytosis, Newborn infants, Dehydration, Dengue haemorrhagic fever, and other conditions associated with haemoconcentration.

References

[1] Letsky, (1999). TheHaematological System. *Clinical Physiology in Obstetrics*: 71– 110.

[2] Am, 1977, International Committee for Standardization. Recommendation of measurement of erythrocyte sedimentation rate of human blood; *68*: 505-507.

[3] Imafuku*et al*., *1998,* Automated measurement of erythrocyte sedimentation rate and its relation to red blood cell concentration and plasma proteins; *40*: 27-32.

[4] AlFadhli *et al.,* 2005, Comparison of erythrocyte sedimentation rate measurement by the automated SEDI system and conventional Westergreen method using the Bland and Altman statistical method. *MedPrincPract*; *14*: 241-244.

[5] Plebaniet al., 1998, The TEST 1 automated system: a new method for measuring the erythrocyte sedimentation rate. *Am J ClinPathol*. *110*: 334-340.

[6] Liu et al.,, 2013,"Preliminary Case-control Study to Evaluate Diagnostic Values of C-Reactive Protein and Erythrocyte Sedimentation Rate in Differentiating Active Crohn's Disease From Intestinal Lymphoma, Intestinal Tuberculosis and Behcet's Syndrome.". The American journal of the medical sciences. 346 (6): 467–72. *PMID 23689052*. *doi*: *10.1097/MAJ.0b013e3182959a18*.

[7] Curvers *et al.,* 2010. Evaluation of the Ves-Matic Cube 200 erythrocyte sedimentation method: comparison with Westergrenbasedmethods. *American Clin Pathol.* 134 (4):

653-60. doi: 10.1309/AJCPMEWW62BGQHJH.

[8] Jou, 2012, Erythrocyte Sedimentation Rate (ESR)-Laboratory Hematology Practice. 638-646. Blackwell Publishing Ltd.

[9] NCCLS, *1988,* Reference Procedure for Erythrocyte Sedimentation Rate (ESR) Tes*t; Approved Standard* (2nd edn., H2-A2. Villanova, PA: NCCLS).

[10] Sezer S, Yilmaz FM, Kaya O, et al. Evaluation of VesMatic Cube 200 for erythrocyte sedimentation rate determination. J Clin Lab Anal 2013; 27: 367-372.

[11] Gülfer Öztürk, Şeyda Özdemir, Aynur Altuntaş, Sümeyya Akyol, Erdem Bulut, Gönül Erden, İsmail Temel (2014). Comparison of two different methods for the determination of erythrocyte sedimentation rate. *Journal of Clinical and Experimental Investigations* 5 (3): 371-375 doi: 10.5799/ahinjs.01.2014.03.0422

[12] Reza Hashemi, Alireza Majidi, Hassan Motamed, Afshin Amini, Fares Najari, Ali Tabatabaey. (2015). Erythrocyte Sedimentation Rate Measurement Using as a Rapid Alternative to the Westergren Method. *Emergency* 3 (2): 50-53

[13] Jou J. M, Lewis S. M., Briggs C., Lee, De la salle B S. ‐ H.., McFadden S. (2011). ICSH review of the measurement of the erythocyte sedimentation rate. *Internal Journal of Laboratory Hematology.* 33 (2).125-132

[14] Kratz A., Plebani M., Peng M., Lee Y. K., McCafferty R., Machin S. J., (2017). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. *Internal Journal of Laboratory Hematology. 39: 448–457. DOI: 10.1111/ijlh.12693*

[15] Alexy T, Pais E, Meiselman HJ. (2009). A rapid method to estimate Westergren sedimentation rates. *Rev Sci Instrum*. 80 (9): 096102. doi: 10.1063/1.3212564.