



Original Article

Comparison of Micro-ESR Method with Westergren Method for Determination of Erythrocytes Sedimentation Rate

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Abstract

Background: Erythrocyte Sedimentation Rate (ESR) is an important diagnostic tool used for diagnosing and monitoring various disease processes. The most important method of ESR estimation is gold standard Westergren method using 3.8% sodium citrate in the ratio of 4:1. Though many other methods are available for ESR estimation not many studies have been done in this regard. This study was done with an aim of comparing gold standard Westergren ESR method with micro ESR method, Modified Westergren ESR method (K3 EDTA using blood in Dilution 0.85% sodium chloride by ratio 4:1) and ESR method using EDTA without blood dilution.

Material and Methods: The present study was done in the haematology section of central diagnostic laboratory services of a tertiary care hospital in Karnataka from November 2018 to July 2019 on blood samples from 110 adult patients. The mean values of ESR estimation done by using blood collected with 3.8% sodium citrate in a ratio of 4:1 was compared with ESR estimated through microesr method, ESR estimated using blood collected in K3 EDTA with dilution with normal saline and ESR estimated using blood collected in K3 EDTA without dilution. The Mean, Standard Deviation and confidence interval was measured using SPSS 22IBM version. Paired t-test was used for calculation of p value, which if $< .001$ was considered statistically significant.

Results: Maximum number of cases were in the age group of 21-40years (70%). 40.9% cases were males and 59.1% cases were females. Out of 110 cases the blood sample 75 cases were normal and 35 cases are abnormal (gold standard method). The comparison of mean values obtained between gold standard method micro ESR methods, undiluted EDTA. While the mean values when compared between gold standard ESR estimation with EDTA blood with normal saline dilution had not much significant difference.

Conclusion: In this study ESR estimation by dilution of EDTA collected blood with normal saline was as good as gold standard Westergren method. It is easy to perform, needs one single vacuitainer and doesnot need any sophisticated instruments. Hence they can be used interchangeably.

Keywords: Westegrens Method, Micro ESR Method, K3 EDTA Dilution.

Introduction

Erythrocyte sedimentation rate (ESR) provides valuable information in screening, monitoring, therapeutic response and follow up of

numerous disease. Its estimation is one of the most commonly requested laboratory tests by physicians and is an important part of diagnostic criteria of critical diseases such as tuberculosis, giant cell arteritis and rheumatological conditions.^{1,2}

Historically, Westergren method and Wintrob's method were commonly used for estimation of ESR. Though Wintrob's method is rarely preferred, Westegren's method and its modifications are still widely used. However, some of the drawbacks of the conventional Westergren method using 3.8% sodium citrate (4:1) are it a time consuming procedure, requires relatively large volume of blood and needs dual sample collection if both

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Hemalatha & Shwetha. ESR estimation by various methods.

complete blood counts and ESR estimation is requested.³ Hence this method may not be effective in critically ill patients who requires multiple samplings, infants and neonates. Reliable, faster, accurate, simple sample collection methodologies for ESR determination are the need of hour. To overcome these drawback of conventional Westergren method, several new techniques and methods such as micro-ESR method, centrifugation method, and automatic ESR analyser method are being evaluated by many. However in resource deficit countries many laboratories are still using the modifications of gold standard Westergren method of ESR estimation.

Aims and Objectives

1. To compare micro ESR method with gold standard reference Westergren ESR method (3.8% sodium citrate).
2. To compare modified Westergren ESR method (K3 EDTA using blood Diluted with 0.85% sodium chloride in ratio of 4:1) with reference Westergren ESR method (3.8% sodium citrate).
3. To compare Westergren ESR method (undiluted K3 EDTA without dilution) with reference Westergren ESR method (3.8% sodium citrate).

Materials and Methods

The present study was done in the haematology section of Central Diagnostic Laboratory Services of a tertiary care hospital in Karnataka from November 2018 to July 2019.

Sample size was calculated as 110 based on the differences in results observed in a study by kratz A et al. between westergren ESR and icro ESR method. Keeping in mind atleast 10% of difference with 80% power and 1% of alpha error.³

Before starting the study Ethical clearance was taken from the institutional Ethical Committee. After taking informed consent from patients, procedure, purpose for blood collection and examination was explained to the patient. Venous blood was collected into 3.8% sodium citrate and K3 EDTA tube, capillary blood through needle prick was collected for Micro-ESR method. All the samples collected were bar coded and transported to

haematology section for processing as per our laboratory policy. All the tests were performed within 4 hours of blood collection.

- a. A westergren ESR Method (with 3.8% sodium citrate) was considered as a reference method (ICSH) and was performed by collecting 1.6ml of blood in black vacutainer which contains 0.4ml of 3.8% sodium citrate anticoagulant (blood to citrate ratio 4:1).
- b. Micro ESR method was done with small amount of blood (0.2ml) taken from fingertip.
- c. For modified Westergren ESR method (with K3 EDTA) 2ml of blood in lavender vacutainer which contains 3.6mg K3 EDTA anticoagulant was used.
- d. Westergren ESR method (K3 EDTA and diluent 0.85% sodium chloride) - 1.6ml of blood collected in 3.6mg K3 EDTA anticoagulant was diluted with 0.4ml of 0.85% sodium chloride. (K3 EDTA+ 0.85% sodium chloride in 4:1 ratio). All the values were expressed in millimetre at the end of one hour.

The Quality assurance was maintained by following the standard operating procedure, for collection of blood samples and performing the test as early as possible. Further, readings were taken by both the authors and all the values obtained were documented in proforma. The standard reference range was used to categorise all the values as normal and abnormal.

The values obtained were analysed using SPSS 22 software for quantitative measure presented by Mean, Standard Deviation and confidence interval. To compare the methods of correlation coefficient paired t-test was used for calculation. p value < .001 was considered statistically significant.

Results

Total of 110 consecutive cases was included in our study. Maximum number of cases were in the age group 21-40years age (70%). and least number of cases belong to age more than 60 years (10.9%). 40.9% cases were males and 59.1% cases were females. Out of 110 cases and 75 cases showed normal values 35 cases while abnormal. Values obtained by all three show methods were compared with the gold standard method.

Table 1: shows the comparison of cases between normal and abnormal

Range	Gold standard	Westergren method		
	3.8% sodium citrate	Micro ESR method	EDTA	EDTA+Dilution
Normal range	75	80	69	83
Abnormal range	35	30	41	27
Total cases	110	110	110	110

Table 2: comparison of mean values, Std deviation error mean and P value among three groups

		Mean	Std. Deviation	Std. Error Mean	t - test	P values
Pair 1	3.8% Sodium Citrate with Micro-ESR -	3.718	7.936	.757	4.914	<0.001*
Pair 2	3.8% Sodium Citrate with Undiluted EDTA	-6.900	6.488	.619	-11.155	<0.001*
Pair 3	EDTA 3.8% Sodium Citrate - with EDTA+Dilution	-.355	1.595	.152	-2.332	0.022

For statistical purpose mean and standard deviation was calculated and values were compared using the paired t test. The comparison of mean values obtained between gold standard 3.8% sodium citrate and Micro ESR method reached statistical significance (t-test 4.914, p value <0.001). Significance was also seen when comparing mean values between 3.8% sodium citrate and undiluted EDTA. Hence the difference in the mean values between the gold standard 3.8% westergrens ESR estimation, when compared with micro ESR method and ESR estimation with EDTA blood without dilution was high hence they cannot be used interchangeably. While the mean values between gold standard ESR estimation with EDTA blood with dilution did not show significant difference hence can be used.

Discussion

Erythrocyte sedimentation rate estimation is one of the most commonly requested laboratory test used by the treating physician as a guiding tool to aid the diagnosis, management and follow up of clinical conditions. In order to achieve uniformity in ESR estimation, in 1988 ICSH based on its study on ESR validation procedure proposed Westergren method using 3.8% sodium citrate anticoagulant (or) (black) vacutainer tube as a Gold Standard method for ESR estimation.

In our study the values obtained from other method were compared with values obtained using gold standard 3.8% sodium citrate ESR method. However for practical purpose such as the convenience of use in primary health care centres, requirement of more blood, difficulty in filling the ESR tube and difficulty in doing other tests such as complete blood counts using blood collected in 3.8% sodium citrate blood, many laboratories across the world use other methods of ESR estimation.³

In order to look into these drawbacks we collected blood from 110 patients visiting our medical

outpatient services who were willing to volunteer for our study. And three methods mentioned above were compared.

Many studies have recommended use of EDTA blood after diluting in normal saline in a ratio of 4:1. Since ESR estimated using EDTA only may show higher values, dilution with normal saline was recommended. Some other studies have refuted this theory and suggested using EDTA blood directly for ESR estimation. It is because by using undiluted blood anticoagulated with K₃EDTA per analytical mistakes due to partially coagulated specimen or small clots, altered blood diluent ratio can be nullified.^{4,5,6}

Even though as per the 2011 ICSH guidelines and recommendations modifications using either whole blood anticoagulated with EDTA and later diluted with trisodium citrate or saline in 4:1 dilution ration in westergrens pipettes, the reliability and reproducibility of the test result is not well established.⁶ However in our study the mean values obtained by ESR estimated using diluted EDTA blood and 3.8% sodium citrate blood did not show much difference in the values. both methods were comparable and use of this method might help in saving resources, non-requirement of dual collection of blood. However technical factors such as dilution factors, availability of adequate blood for ESR estimation must be kept in mind before following this method.

While comparing micro ESR method with westergren method. Many studies have shown both conflicting and agreeable findings between both these methods. In a study on 108 blood samples, author concluded that micro ESR method was faster, cheaper, more reliable, and precise tool for ESR measurement especially in the Emergency Department.²

In another study with Accu-tech micro method, it was described that micro ESR method was simple to perform, reproducible and required only

Hemalatha & Shwetha. ESR estimation by various methods.

0.2 ml blood. It correlated with the reference Westergren method, can be applied to either venous or capillary blood and is a completely disposable system allowing safe handling of samples and can be used in paediatric population and very old patients.^{1,7} However, in another study using micro ESR method the author has suggested revision in the reference values in paediatric population for ESR estimation, especially patients with neonatal sepsis.⁸

Hence factors such as the duration of time, the type of patients, should be looked into before using this method. No clear guidelines pertaining to duration of the test, length of the capillary tube, the type of coating in the tube, use or disuse of centrifugation has to be addressed before finalising this method.

The mean values between ESR estimated using EDTA without dilution and 3.8% sodium citrate were very different. Other studies have also opined that both tests yielded significant results. A different reference range has to be established for estimation of ESR using undiluted blood.^{5,9}

In view of undiluted EDTA blood requiring only limited amount of blood, apart from saving in terms of sodium citrate and additional non-vacuum blood collection vial, newer reference methods were suggested.

Conclusion

In this study ESR estimation by dilution of EDTA collected blood with normal saline was as good as gold standard Westergren method. It is easy to perform, needs one single vacutainer and does not need any sophisticated instruments.

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