Journal of Associated Medical Sciences 2019; 52 (1): 33-36

JAMS
Journal of Associated Medical Sciences

Thai-Journal Citation Index Centre (TCI) & ASEAN Citation Index (ACI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

Rapid alternative methods for erythrocyte sedimentation rate measurement

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ARTICLE INFO

Article history:
Received 14 September 2018
Accepted as revised 19 October 2018
Available online 30 October 2018

Keywords:
Erythrocyte sedimentation rate, Westergren method, Wintrobe with centrifugation, capillary tube with centrifugation

ABSTRACT

Background: Erythrocyte sedimentation rate (ESR) measurement is the most widely used in laboratory test to assess an inflammation or acute phase response.

Objectives: To introduce two rapid alternative methods for ESR measurement.

Materials and methods: ESR was performed in 449 blood samples using Westergren, Wintrobe with centrifugation (Win-C) and capillary tube (Cap-C) with centrifugation methods.

Results: Win-C and Cap-C methods were significantly correlated with Westergren method by using Spearman rank correlation. Both Win-C and Cap-C methods were demonstrated the good % of sensitivity, % of specificity, % of positive predictive value, % of negative predictive value and cut-off point by using the receiver operating characteristic (ROC) curves analysis.

Conclusion: Both methods demonstrated acceptable agreement with the conventional ESR measurements and appear to be a faster, easier and safety.

Introduction

Erythrocyte sedimentation rate (ESR) measurement is one of the most widely used laboratory test to assess an inflammation or acute phase response. The ESR phenomenon is the combination of aggrega¬tion, precipitation, and packing of erythrocytes. These are included with fibrinogen, immuno¬globulins, amount and shape of erythrocytes facilitation.[1] Although it is not considered a specific diagnostic test but useful for the monitoring and follow-up for certain groups of patients, such as rheumatoid arthritis, temporal arthritis, polymyalgia rheumatica, and Hodgkin' s disease. Besides it is used in prognostic value in acute coronary syndrome and stroke. The Westergren method has been used and proposed as a conventional ESR measurement by several agencies.[2] In general, anticoagulant and diluted blood samples were used in the Westergren tube, which determines the sedimentation of erythrocytes after 1 hour at room temperature in a strictly vertical by the mounted tube of defined length and bore size. The distance of the erythrocytes fallen was measured in millimeters (mm) as ESR result. This manual method has many processes in the pre-analytical and analytical step which had many factors that can affect ESR results and high biohazard risk. Thus, many research studies have tried to look the better suitable and speed methods for the modern clinical laboratories.

Nevertheless, a number of modifications of the Westergren method are now available including smaller sample volume, less manual manipulations, shorter testing times, automation, and interfacing with the laboratory information system. We aim to demonstrate and propose our two rapid alternative ESR measurement methods.

Materials and methods

Blood samples

A total of 449 blood samples from the participants in Cardiovascular and Diabetes Prevention in Elderly Project were used to test ESR examination during October 2012-December 2014. We did not have any specific inclusion and exclusion criteria. These blood samples were from 204 men (77 with aged <50 yrs, 127 with aged ≥50 yrs) and 245 women (121 with aged <50 yrs, 124 with aged ≥50 yrs). All blood samples were collected by sterile venipuncture of

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doi: 10.14456/jams.2019.6
E-ISSN: 2539-6056

K3-EDTA vacuum tubes (Vacutainer, Becton Dickinson, UK) and carried out all ESR measurements within 4 hr. The study protocol was approved by the Ethics Committee of the Naresuan University.

Erythrocyte sedimentation rate measurements

(i) Westergren Method:

In the beginning, we performed the conventional ESR measurement by using Westergren method in our laboratory, 3 by using Westergren tube, a 200-millimeters (mm) long vertically graduated glass tubes and 2.55 mm in diameter with open at each end (Curtin Matheson Scientific, Houston, TX). Briefly, 1 mL of K3-EDTA blood samples were diluted to 4:1 with 0.9% normal saline solution (250 µL) and manual gently inversion mixed and transferred into the Westergren tube to reach 200 mm mark and vertically into supporting stand at room temperature. ESR result, red blood cell sedimentation was read and recorded as the mm mark after 1 hr (mm/hour).

(ii). Wintrobe with centrifugation (Win-C) method:

This method used the Wintrobe tube and centrifugation force for ESR measurement. Briefly, K3-EDTA undiluted blood sample was filled into the Wintrobe tube (with 0 to 100 mm mark) and centrifuged at 500 rpm for 3 min at room temperature. After that the distance of the fallen RBC and plasma front was immediately measured and recorded (mm mark on the Wintrobe tube).

(iii). Capillary tube with centrifugation (Cap-C) Method:

Briefly, K3-EDTA undiluted blood samples were gently mixed and drawn into the micro-capillary tubes (glass plain blue tip) and sealed with the sealing clay at the end of the tube. Then, capillary tubes were centrifuged at 500 rpm for 3 min at room temperature. After that the distance of the fallen RBC and plasma front was immediately measured and recorded with the ruler as millimeter.

Both Win-C and Cap-C methods were termed the sedimentation index according to the study of Alexy et al.[4] that using centrifugation force as the special hydrodynamic force for increased the sedimentation rate. Westergren methods, Win-C and Cap-C methods were performed by the same time and same researcher for all blood samples.

Statistical analysis

All results were demonstrated as median and interquartile. Spearman rank correlation was used to test the correlation of Win-C and Cap-C with Westergren methods. The receiver operating characteristic (ROC) curve were analyzed the comparison results of these 3 methods. A ROC curve is a plot between sensitivity (Y-axis) versus false positive (X-axis), obtained for different cut-off points. Areas under the curve (AUC) of the ROC curves and 95% confidence intervals (CI) were evaluated as the accuracy of diagnostic measurement. A discriminate analysis was performed to identify these parameters that provided the best differentiation between Win-C and Cap-C methods. Greater AUC of the ROC curve indicated better markers of the study. In general, an AUC of a ROC of <0.5 suggests no discrimination, whereas a maximal AUC of a ROC of 1 suggests outstanding discrimination.[5] The $p<0.05$ (two tailed) were considered as significant.

Results

The results of ESR measurement with Westergren method were categorized to 202 (44.7%) normal-ESR and 247 (55.3%) Elevated-ESR as demonstrated in Table 1 with reference ranges of the method. Results of ESR measurement by both Win-C and Cap-C methods were demonstrated in Table 2. The results of ESR measurement by Win-C and Cap-C methods were significantly correlated with Westergren method (r=0.929, $p<0.001$ and r=0.859, $p<0.001$), respectively. Both Win-C and Cap-C methods were demonstrated discrimination as AUC>0.5 and good % sensitivity, % specificity, % positive predictive value (PPV) and % negative predictive value (NPV). The AUCs, optimal cut-off point, % sensitivity, % specificity, %PPV and %NPV of Win-C and Cap-C methods were demonstrated in Table 3.

Table 1 Demonstration the results of 449 ESR measurements by using Westergren method.

ESR results	Samples (%)
Normal-ESR	202 (44.7%)
Elevated-ESR	247 (55.3%)
* Normal reference range of men age	
<50 yrs.	0-15 mm/hr
≥50 yrs.	0-20 mm/hr
*Normal reference range of women age	
<50 yrs.	0-20 mm/hr
≥50 yrs.	0-30 mm/hr

Nor-ESR: normal ESR results, Ab-ESR: higher ESR results

Table 2 Demonstration of median, interquartile, minimum and maximum results of 449 ESR measurements by each method.

Methods	ESR results		
	Median (interquartile)	Minimum	Maximum
Westergren (mm/hr)	24.0 (12.0–50.5)	1.0	155.0
Win-C (mm)	36.2 (28.0–49.0)	2.0	77.0
Cap-C (mm)	46.7(40.0–54.3)	4.0	73.0

Table 3 Demonstration of the cut-off points, AUC, %sensitivity, %specificity, %PPV and % NPV of the ESR measurements with Win-C and Cap-C methods.

Methods	Group (sex, age)	n	cut-off (mm)	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Win-C	Men <50 yrs.	77	0-28	0.886	87.8	86.1	87.9	84.1
	Men ≥50 yrs.	127	0-37	0.918	90.7	82.1	94.4	70.9
	Women <50 yrs.	121	0-35	0.879	85.9	72.0	80.0	85.9
	Women ≥50 yrs.	124	0-40	0.872	88.8	77.8	79.1	100.0
Cap-C	Men <50 yrs.	77	0-40	0.848	80.5	71.2	79.1	77.8
	Men ≥50 yrs.	127	0-47	0.863	80.1	78.6	88.0	65.4
	Women <50 yrs.	121	0-45	0.829	79.1	78.0	79.3	84.9
	Women ≥50 yrs.	124	0-50	0.825	80.5	79.4	64.9	95.8

AUC: area under the curve, PPV: positive predictive value, NPV: negative predictive value.

Discussion

ESR is widely used as a clinical guide to aid in the diagnosis, follow up and management in rheumatoid arthritis, temporal arthritis, osteomyelitis and clinical significant in stroke, coronary artery disease, prostate cancer, tuberculosis and Hodgkin disease.[6-8] Generally, ESR measurement may affect by age, race, and blood storage which importance in the clinical conditions. Sedimentation is increased in anemia (megaloblastic higher than iron-deficiency anemia) while polycythemia inhibits sedimentation. Red cells morphology (spherocytosis, acantocytosis, and sickle cells) are also affected sedimentation rate,[8,9] and combination with hemoglobin, lipid concentrations, plasma proteins ratio and plasma pH.[10]

Many new methods for ESR measurement have been developed and proposed for clinical laboratories.[4, 6, 11] These new methods are shorter time testing and guarantee safety both in automated and manual systems. Both Win-C and Cap-C methods were performed with Wintrobe tube and micro-capillary tubes which required less blood sample (suitable for pediatric use), advantages of speed, safety and uniform specimen handling. Centrifugation force in both Win-C and Cap-C methods was used as the special hydrodynamic force accelerated the erythrocytes sedimentation. Win-C and Cap-C methods were suitable for the high workload clinical laboratories and emergency laboratories. Consideration in the limitations of this present study, no strict inclusion and exclusion criteria were implemented. All results of ESR measurements were carried out in one time and we do not estimate the reproducibility of the results.

Conclusion

Our rapid alternative ESR measurement, Win-C and Cap-C methods appear to be the faster, reliable and safety. Win-C may suitable for routine and emergency laboratory while Cap-C may suitable for Pediatric Division with a smaller sample volume.

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