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SHORT COMMUNICATION

SIGNIFICANCE OF DILUTION ERRORS IN THE MEASUREMENT OF ERYTHROCYTE SEDIMENTATION RATE

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Abstract: Erythrocyte sedimentation rate test (ESR) by the Westergren method involves the use of a blood sample, anticoagulated and diluted 4:1 with sodium citrate solution. Dilutions are made during sample collection. However, the method invariably leads to errors in the dilutions. This study was done to assess the effects of these dilution errors on the results of the ESR test. The results indicate that the under diluted samples sedimented faster and the over diluted samples slower than standard dilutions, resulting in significant errors in the ESR readings.

Key words: Blood, dilution errors, ESR.

INTRODUCTION

Erythrocyte sedimentation rate (ESR) is an empirical test widely used in clinical medicine as a non specific reaction giving information of general character, with the same usefulness as body temperature, pulse rate or leucocyte counts.[1] Although a normal ESR cannot be taken to exclude organic disease,[2] it is a measure of the presence and severity of inflammatory and other morbid processes. [1] It also provides means for monitoring the progress and response to therapy in chronic diseases.[3] At times it may be more sensitive than other means, in detecting an abnormal state and may draw attention to an otherwise occult disease.[1]

ESR test measures the sedimentation of aggregated red cells in their native plasma. It is one of the commonest investigations carried out in a pathology laboratory. In our laboratory about 40% of the daily haematological investigations are ESR measurements. It is a test which is easy to perform needing only simple equipment and little technical expertise. However as there are no recognised standard samples available for monitoring the test, the reliability and reproducibility of the results depend on the use of correct methodology.

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The Westergren method[4] is commonly used for ESR measurements as recommended by the International Committee for Standardisation in Haematology.[5] This method essentially involves the use of a standardised tube and a diluted sample of venous blood. 3.8% sodium citrate solution is used both as the anticoagulant and the diluent for the blood sample. The optimum dilution to be used is one volume of diluent to four volumes of anticoagulated blood.[2] The diluted blood is then withdrawn into the tube and allowed to stand upright. Readings are taken at the end of 1 h. The ESR is not influenced by meals or by a circadian rhythm.[3] If the tubes are kept vertical[3] and away from direct sunlight,[6] the only significant variable which influences the results is the dilution of the blood samples.[7] This however cannot be controlled easily because the dilutions are made during the process of sample collection and the inherent faults in the method commonly employed for this purpose invariably lead to dilution errors. The usual practice is to withdraw 1.6 ml (4 volumes) of blood directly into a syringe containing 0.4 ml (1 volume) of citrate.[2] Dilution errors constantly occur at this stage as it is not easy to withdraw an exact amount of blood into a syringe already containing a solution. Difficult venesections, particularly in children result in markedly incorrect dilutions. The alternative method of adding the required amount of blood into a sample bottle containing the citrate solution also has its drawbacks because of the difficulty encountered in delivering an exact amount of blood from a syringe.

This study was undertaken to observe the influence of the errors in dilution of blood samples on the results of the ESR measurements. 258 blood samples were tested. The solid anticoagulant sequestrine was used to anticoagulate the blood in this study in order to eliminate the dilution errors that occur at the stage of sample collection.[8] It has been shown that the sedimentation rate of blood anticoagulated with sequestrine, appropriately diluted, is the same as that of blood anticoagulated and diluted with sodium citrate as for the orthodox Westergren technique.[9,10] The dilutions should be made immediately before the test and within 2 h of collecting the blood sample, using 3.8% sodium citrate or normal saline.[2] This procedure was adopted and accurate dilutions were made with sodium citrate solution, using graduated pipettes. Three sets of dilutions namely the standard dilution, an under dilution and an over dilution were made from each blood sample and the ESR readings of these dilutions were taken at the end of 1 h.

In a similar earlier study done on 50 blood samples, Sreeharan[7] showed that overdilution of the sample by using an extra 0.2 ml citrate resulted in a marked lowering of the ESR readings.

METHODS AND MATERIALS

Ethical clearance was obtained for the procedure. The objectives were explained to patients who came for ESR tests to the clinical pathology laboratory and an extra 3 ml blood was taken from those who gave their consent for the study. Sample bottles were prepared so as to contain 7.5 mg sequestrine (EDTA), and the total volume of 5 ml of blood collected from the patients were added into these and mixed well. Three dilutions of these blood samples were then made with 3.8% sodium citrate without delay, using graduated pipettes, in the following manner:

Dilution A : 0.3 ml citrate + 1.2 ml blood
(Standard dilution - 1 part citrate and 4 parts blood)
Dilution B : 0.2 ml citrate + 1.4 ml blood
(Under dilution - 1 part citrate and 7 parts blood)
Dilution C : 0.7 ml citrate + 0.7 ml blood
(Over dilution - 1 part citrate and 1 part blood)

ESR tests were set up with the three dilutions A,B and C made from the blood samples collected from each patient. Verticality of the tubes was ensured and the tubes were kept away from direct sunlight. Readings were taken at the end of 1 h.

RESULTS AND DISCUSSION

258 blood samples were tested. The readings obtained were divided into 5 groups depending on the ESR values of the standard dilution (dilution A). The mean ESR reading of each group for the three sets of dilutions A,B and C were calculated. The deviation of the mean readings of the under and over dilutions from the mean reading of the standard dilution were also calculated. (Table 1).

The results indicated that significant differences occur in the ESR readings due to errors in the dilution of the blood samples used.

The under diluted samples sedimented at a faster rate. The over diluted samples sedimented at a much slower rate compared to the standard dilutions consistent with previous observations.[7] The ESR readings within the normal range (<15mm) appeared to be only slightly affected. In the >100 mm group the mean increases in the readings of the under diluted samples were not so marked as in the other groups because the sedimentation is usually at its end stage when these high readings are reached. The lower number of samples used in this group may also be a contributory factor. However the mean decreases in the readings of the over diluted samples in this group were in agreement with those of the

other groups. Particular care should therefore be taken to ensure correctness of the dilutions, when blood samples are collected for ESR measurements.

Table 1: Comparison of ESR readings of under and over dilutions with those of the standard dilution.

	Groups according to ESR readings of dilution A at 60 min				
	gp 1	gp 2	gp 3	gp 4	gp 5
	15mm (n=55)	15-49mm (n=77)	50-79mm (n=52)	80-100mm (n=50)	>100mm (n=24)
Dilution A: Mean value	8 mm	25 mm	60 mm	90 mm	120 mm
Dilution B: Mean value	13 mm	36 mm	73 mm	106 mm	128 mm
Mean difference from A	+ 5 mm	+ 10 mm	+13 mm	+17 mm	+ 9 mm
Range of diff. from A	+1 to +13mm	+7 to +24mm	+7 to +20mm	+10 to +26mm	+5 to +12mm
Dilution C: Mean value	3 mm	5 mm	9 mm	14 mm	58 mm
Mean difference from A	- 6 mm	- 20 mm	- 51 mm	- 75 mm	- 61 mm
Range of diff. from A	-1 to -11mm	-8 to -40mm	-38 to -65mm	-57 to -88mm	-50 to -72mm

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