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An Investigation into the Preanalytical and Analytical Factors Influencing the Determination of the Erythrocyte Sedimentation Rate

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Abstract: ***This study aims to evaluate the variation in Erythrocyte Sedimentation Rate (ESR) due to various preanalytical and analytical factors. The preanalytical variables examined include the anticoagulant to blood ratio, storage time of blood samples(refrigerated), and the tilt angle of the ESR tube. Additionally, the impact of time delay between blood collection and ESR determination is analyzed. By systematically assessing these factors, the study seeks to identify critical variables that influence ESR measurements, thereby enhancing the accuracy and reliability of ESR as a diagnostic tool. The findings are expected to provide valuable insights for standardizing ESR testing protocols and minimizing potential errors in clinical settings.***

Keywords: Erythrocyte Sedimentation Rate, anticoagulants, storage time, tilt angle

1. Introduction

The rate at which red blood cells sediment in an hour is known as the erythrocyte sedimentation rate, or sedimentation rate (ESR). It is a typical hematology test that measures inflammation in an indiscriminate manner. Traditionally, anticoagulated blood was used for the test, and the red blood cell fall rate was recorded and expressed in millimeters per hour (mm/hour) using an upright tube called a Westergren tube [1]. The Westergren method, which uses Westergren tubes, is a frequently used technique to measure erythrocyte sedimentation rate. Additional techniques including the Wintrobe and micro-ESR approaches were also employed. It has been carried out automatically ever since automated analysers were introduced into clinical laboratories.

Increases in inflammation, pregnancy, anemia, autoimmune diseases (including lupus and rheumatoid arthritis), infections, some kidney diseases, and certain malignancies (like multiple myeloma and lymphoma) are all associated with elevated ESR (erythrocyte sedimentation rate).

Polycythemia, hyperviscosity, sickle cell anemia, leukemia, low plasma protein (from renal or hepatic disease), and congestive heart failure all resulted in a reduction in the ESR score. Despite the fact that elevated immunoglobulin levels often result in an increase in the ESR, their hyperviscosity can cause an ESR reduction at very high levels. This is more likely to occur with paraproteins of the IgM class and, to a lesser degree, the IgA class. In women, the basal ESR is marginally higher. Although it aids in diagnosis, ESR is not a diagnostic tool in and of itself.

2. Method

a) Ratio of anticoagulant and blood

A sample of citrate was taken from human subjects. One sample was taken while keeping the blood to anticoagulant ratio at 1:4. By altering the anticoagulant blood ratio to 1:9 and 1:2, more samples were gathered. 1.8 ml of blood and 0.2 ml of 3.8% trisodium citrate anticoagulant were drawn into a tube to create a 1:9 ratio. A 1:2 anticoagulant blood ratio was obtained by combining 0.5 milliliters of 3.8% trisodium citrate with one milliliter of blood.

Using the Westergren method, the erythrocyte sedimentation rate was calculated for each of the three samples. The results were statistically evaluated and compared. To ascertain the impact of variations in the anticoagulant to blood ratio, thirty samples were examined. [3]

b) Time delay

Blood and anticoagulant were collected in a 1:4 ratio from volunteers using a 2 cc glass container filled with trisodium citrate. The sample was homogeneously mixed. The Westergren method was used to calculate the ESR. The ESR of the same tube was then once more measured at 4- and 6-hour intervals. This was done to reduce the amount of time that the sample processing and specimen transportation took. A statistical comparison was made between the results obtained after two hours of data collecting and processing. Thirty human volunteer samples were used to examine the impact of time delays.

c) Stored sample

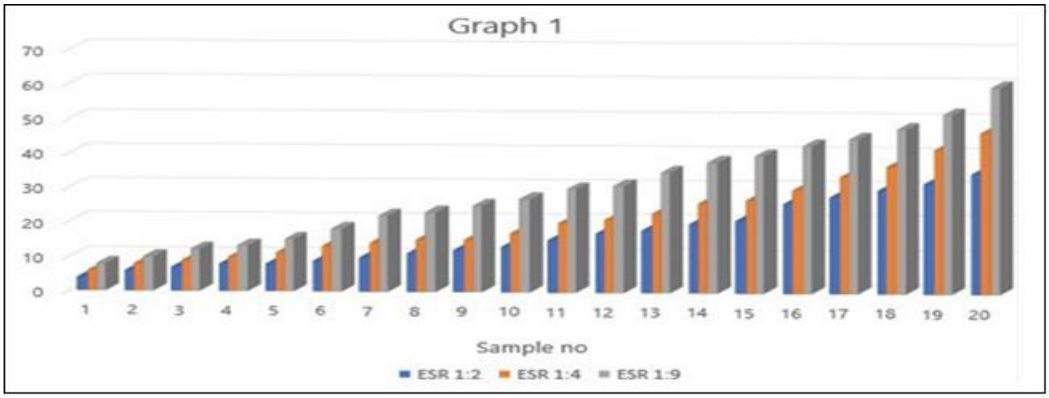
The citrate blood samples were collected from human volunteers. Two samples were collected maintaining the 1:4 ratio of anticoagulant and blood. ESR of each sample was determined by Westergren's method. One sample following the standard conditions maintaining the room temperature at 20-25◦C and ESR determined within 2hrs of blood

collection. Other sample was stored at refrigerated for 24 hours. Then bring to room temperature and determined ESR. The results of stored blood sample were compared with the fresh blood sample.30 samples were analysed to find out the effect of stored sample. The results were statistically analysed.

d) Tilt angle

Throughout the test, the ESR tube needs to be kept upright. Every sedimentation rack that is utilized needs to have a spirit bubble and leaving screws installed. The ESR tube tilting causes more sedimentation. Human volunteers provided samples, which were collected in a 2 ml glass bottle with trisodium citrate and anticoagulant blood in a 1:4 ratio. Blend thoroughly. Tilt the pipettes with blood into 15degree angle, using a stand for pipette after 1 hour read the plasma column.[4]

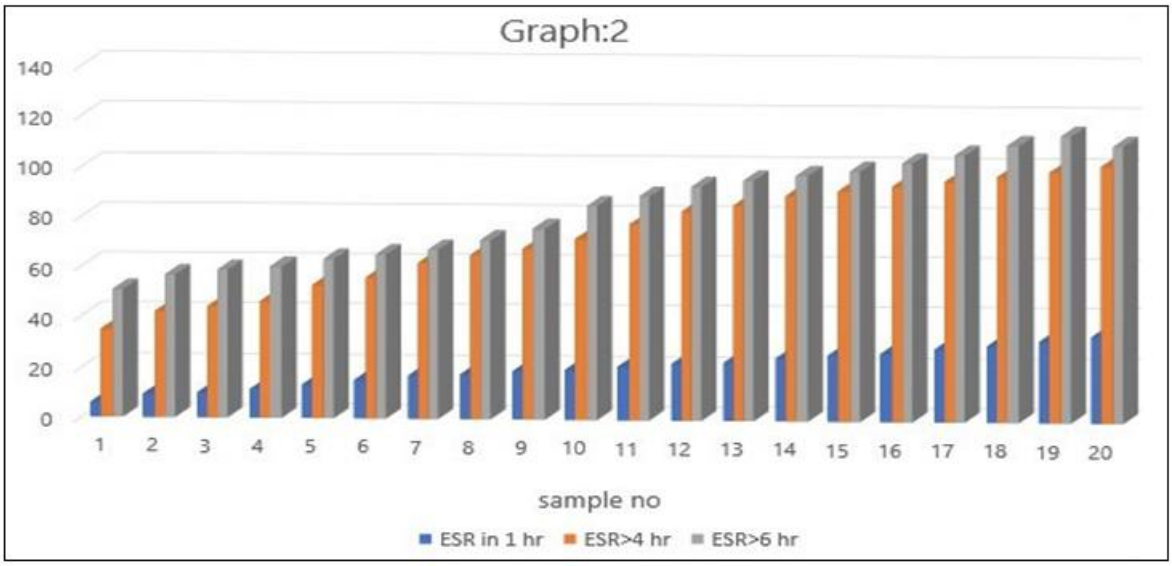
3. Results



Graph 1: Effect of anticoagulant-blood ratio

Table 1: Effect of anticoagulant-blood ratio

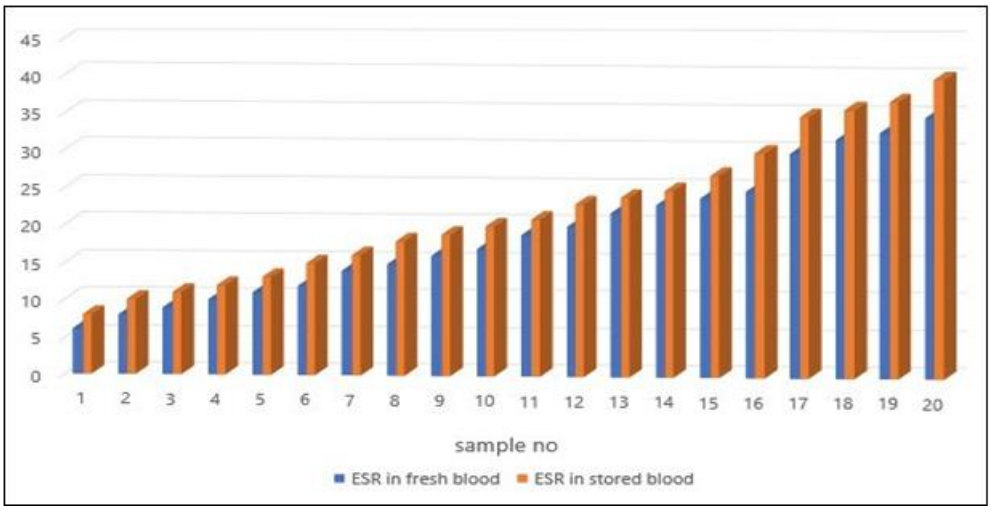
Anticoagulant :blood Ratio		ESR Value		
		Mean	SD	CV
1	1:4	17.7	7.5	42.3
2	1:2	14.2	6.5	45.7
3	1:9	31.3	5.76	543.4



Graph 2: Effect of time delay

Table 2: Effect of time delay

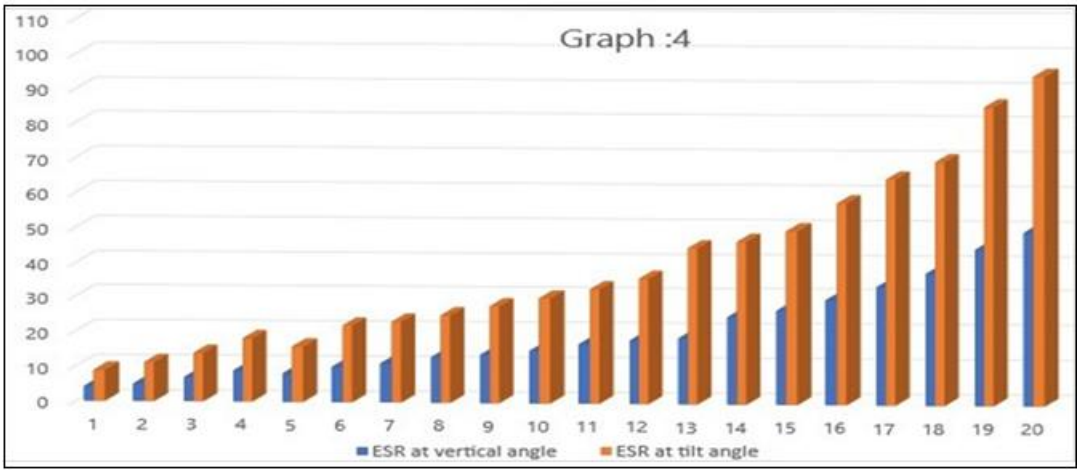
Time delay	ESR value		
	Mean	SD	CV
1 hour	17.7	7.5	42.3
>4hour	71.2	30.1	42.2
>6hour	82.2	35.4	43.06



Graph 3: Effect of stored blood sample

Table 3: Effect of stored blood sample

Blood sample	ESR Value		
	Mean	SD	CV
Fresh blood	17.7	7.5	42.3
Stored blood	28	12.3	45.2



Graph 4: Effect of tilt angle

Table 4: Effect of tilt angle

Angle	ESR value		
	Mean	SD	CV
Vertical angle	17.7	7.5	42.3
Tilt angle	30.5	14.7	48.1

4. Conclusion

In the BSc MLT department of Govt. Medical College, Thiruvananthapuram, thirty samples taken from human volunteers were analysed for four different parameters (preanalytical and analytical variables). The general findings of the various study parameters are;

- Strict adherence to the anticoagulant-blood balance is necessary to maintain the optimal 1:4 ratio.
- It is imperative to prevent any delays in processing the sample. Ideally, the sample should be processed in two hours after it is collected.
- It is best to stay away from the chilled sample. Before testing, the sample should be allowed to come to room temperature if it has been kept in the refrigerator.
- Throughout the test, the tube must remain in its erect, undisturbed position. If the ESR tube tilting operation needs to be repeated.

Due to poor sample quality, ESR testing is particularly prone to errors and misinterpretations. Recommendations for standardising preanalytical and analytical stages must be closely adhered to in order to maximise the precision, consistency, and reliability of ESR testing.

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