



## Hematology

# A COMPARATIVE STUDY OF ERYTHROCYTE SEDIMENTATION RATES OF BLOOD ANTICOAGULATED WITH TRISODIUM CITRATE AND ETHYLENE DIAMINE TETRA ACETIC ACID AMONG PATIENTS WITH MENTAL ILLNESS ATTENDING A TERTIARY CARE PSYCHIATRIC INSTITUTE

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**ABSTRACT** **Background:** An erythrocyte sedimentation rate (ESR) is a nonspecific laboratory investigation, which is not a specific test for diagnosis of any disease. However an increase level of ESR may indicate presence of inflammation, infection, rheumatologic disease, or malignancy. For monitoring the test there has been no accepted standard control sample. Studies are available relating to ESR values of different diseases; however no study was performed to compare the ESR values of blood mixed with TSC and EDTA in the study area. So, this study aimed to compare the ESR values of the two commonly used anticoagulants in patients with psychiatric illness. **Material And Methods:** A cross sectional study was conducted in a tertiary care psychiatric institute of North-East, India during the period of October to December, 2023. A total 100 samples were collected from admitted patients of 25 - 60 years of age with psychiatric illness. Samples were collected in Ethylene Diamine Tetra Acetic Acid (EDTA) and Trisodium Citrate (TSC) anticoagulated vials to determine and compare Erythrocyte sedimentation rate (ESR) by using Westergren method. **Results:** In this study ESR obtained from blood anticoagulated with EDTA shows higher value than the blood anticoagulated with TSC with a mean value of 34.83 mm/hour in EDTA anticoagulated blood as compared to the mean value of 28.14 mm/hour in TSC anticoagulated blood. It shows a difference of 8.03 mm/h with t-value 9.282 which is statistically significant. **Conclusion:** Present study showed ESR value of blood anticoagulated with EDTA is greater than that of blood anticoagulated with TSC with a mean difference of 8.03 mm/h among patient with mental illness. The difference between both the anticoagulant for using ESR estimation is statistically significant. Different reference values may be incorporated for both the anticoagulant (EDTA and TSC) for ESR estimation for accurate report.

**KEYWORDS :** Erythrocyte Sedimentation Rate; Trisodium Citrate; Ethylene Diamine Tetra Acetic Acid; Westergren Method

## INTRODUCTION

Erythrocyte Sedimentation Rate (ESR) measures the rate of settling down of erythrocytes and is a commonly advised non-specific hematology test. The test can be used for monitoring the course of infections, evaluation of acute phase inflammation, malignancy, autoimmune diseases etc.. It can also be used to evaluate response to therapy in tuberculosis, bacterial endocarditis, acute rheumatic fever, rheumatoid arthritis, polymyalgia rheumatica, Hodgkin's diseases etc.<sup>[1]</sup> Increase level of ESR can be observed in various conditions like infections, inflammation, pregnancy, anaemia, auto-immune disorders (like rheumatoid arthritis and lupus), some kidney diseases and some cancers (like lymphoma and multiple myeloma). Decrease level of ESR can be observed in polycythemia, hyperviscosity syndrome, congestive cardiac failure, sickle cell anaemia, hereditary spherocytosis, hypofibrinogenemia, leukemia, chronic fatigue syndrome etc.<sup>[2]</sup> Presence of Immunoglobulins can increase the ESR, however very high levels can decrease it again due to hyperviscosity of the plasma.<sup>[3]</sup> Mild increase level of ESR can be observed in females.<sup>[4]</sup> The most important factor affecting ESR is the composition of plasma. High levels of fibrinogen and acute phase proteins (like C-reactive protein, haptoglobin, ceruloplasmin, alpha 1 antitrypsin etc.) are associated with increase in ESR. Increase proteins in plasma reduce negative charge on the surface of the erythrocytes and reduce the zeta potential (the electric repulsion between erythrocytes) and facilitate rouleaux formation. Inflammation and infection can increase the concentration of fibrinogen in blood which further increases the rouleaux formation.<sup>[5,6]</sup> Elevation of ESR may be greater than 100mm in cases of tuberculosis, severe infection of skin and bone, metastasis, polymyalgia, temporal arteritis, auto-immune diseases and rheumatic fever.<sup>[7]</sup> ESR may be an indicator of progression towards AIDS in HIV seropositive cases.<sup>[8]</sup> Increased level of ESR can also be used as a non-specific sickness index in the elderly. However correlation of elevated ESR in asymptomatic individual is difficult to establish.<sup>[9]</sup> ESR estimation is traditionally performed by placing anticoagulated blood in an upright tube, known as Westergren tube and the distance in which the erythrocytes fall is measured and reported in millimetres after one hour.<sup>[10]</sup> Estimation of sedimentation rates of aggregated red cells in plasma is the main principle of ESR.<sup>[11]</sup> Westergren method is

recommended as a method of choice for ESR estimation by the International Council for Standardization in Hematology (ICSH) because of its sensitivity. The method was described by the ICSH initially which is also adopted and approved Clinical and Laboratory Standards Institute (CLSI). As per the method, venous blood should be collected in vacutainer containing ethylene diamine tetra-acetic acid (EDTA). The sample should be diluted in the proportion of 1 volume of citrate (109mm/L) to 4 volumes of blood and the test should be performed within 4 hours of dilution. A delay of up to 6 hours may be accepted however the sample should be kept at 4°C. Without dilution, EDTA anticoagulated blood can be used up to 24 hours provided the sample is kept at 4°C. After putting the mixed sample up to 200 mm mark of the Westergren tube the tube should be kept vertically for exactly 60 minutes. The height of the clear plasma should be measured and the result should be expressed in mm after one hour.<sup>[12]</sup> Although it is one of the commonest investigations advised in routine practice, for monitoring the test there has been no accepted standard control sample. Uses of correct methodologies are important for the reliability and reproducibility of the test results. Various researches are available relating to ESR values of different diseases; however no study was performed to compare the ESR values of blood mixed with TSC and EDTA in the study area. So, this study aimed to compare the ESR values of the two commonly used anticoagulants in patients with psychiatric illness.

## MATERIALS AND METHODS

This cross-sectional laboratory based study was performed in the Department of Pathology and included 100 samples from patients of 25 to 60 years of age with psychiatric illness and admitted to the Department of Psychiatry of a tertiary care psychiatric institute of North-East, India, from October 2023 to December 2023.

### Inclusion Criteria

1. Routine blood samples which include ESR; 2. Both genders between age group of 25 years to 60 years.

### Exclusion Criteria

Inadequate and clotted samples were excluded

## Enrollment Of Patients And Laboratory Investigation

Written informed consent was taken from the patients or legal guardians in conformation with standard bioethical norms. The study was approved by the Institutional ethics committee. Demographic profile like age, sex and geographic location were obtained by using a pre-designed proforma. A total 3.6ml of venous blood was collected after taking aseptic and antiseptic measures from each patient. 2 ml of blood was for EDTA and 1.6 ml for sodium citrate vials and samples were immediately processed in Pathology laboratory to compare the ESR value by using reference method i.e. Westergren Method as per standard protocol.

## Statistical Analysis

## RESULT

### Socio-demographic -

During the study period 100 samples were collected from patients age 25 to 60 years. So, in male the participants were 68 and in female 32 respectively. (Table-1)

**Table1: Socio-demographic Information Of The Study Of 100 Participants**

| Gender | Frequency | Percent(%) |
|--------|-----------|------------|
| Male   | 68        | 68         |
| Female | 32        | 32         |
| Total  | 100       | 100        |

### Comparison of values of ESR (TSC and EDTA)

The mean $\pm$ SD ESR values of ESR of TSC and EDTA were 28.14  $\pm$  20.069 and 34.83  $\pm$  20.409 mm/h respectively (TABLE 2). Therefore ESR value of EDTA blood is higher than TSC blood from all the variables. The paired sample analysis of t-test showed that anticoagulated blood of TSC and EDTA have statistically significant EDTA were 8.03  $\pm$  5.659 mm/h and the t-value (9.282) (TABLE 3). Therefore, 95% level of significant difference between TSC and EDTA anticoagulants.

### Paired T Test Between TSC and EDTA

**Table 2: Paired Samples**

| Variables | Mean  | Std.Deviation |
|-----------|-------|---------------|
| TSC       | 28.14 | 20.069        |
| EDTA      | 34.83 | 20.409        |

**Table 3 Paired Sample Test**

| variables | Mean  | Std. Deviation | 95% confidence interval of the difference |        | t     | df | significant |
|-----------|-------|----------------|-------------------------------------------|--------|-------|----|-------------|
|           |       |                | Lower                                     | upper  |       |    |             |
| TSC-EDTA  | 6.690 | 7.208          | -8.120                                    | -5.260 | 9.282 | 99 | 0.001       |

\*Level of significance is 0.001 which is less than 0.05 level of significance, so in the given study, it was found that the paired t test is significant

\*Under SPSS version 20 the done for analysis.

## DISCUSSION

This study was mainly done to see the influence of anticoagulants in the results of ESR. Though ESR is not a specific marker for inflammation and other diseases still it is frequently used as a supportive evidence for both evaluation and monitoring of diseases. In this study we have observed that result of ESR obtained from blood anticoagulated with EDTA shows higher value than the blood anticoagulated with TSC. The result of our study was almost similar to the study conducted by Emelike O et al whose final finding stated that the mean  $\pm$  SD ESR value of EDTA anticoagulated blood was higher than the mean  $\pm$  SD ESR value of the TSC<sup>[18]</sup>. In our study mean value EDTA anticoagulated blood is 34.83 mm/h as compared to the mean value of anticoagulated blood TSC is 28.14 mm/h. It shows a difference of 8.03 mm/h with t-value 9.282. Similar to this Getaneh et al. conducted a study comparing ESR on EDTA and TSC anticoagulated samples, in which the mean difference of ESR values between the use of EDTA and TSC anticoagulated blood was 6.91  $\pm$  13.66 mm/h with a t-value of 4.24 ( $P < 0.0001$ ; 95% CI 3.66–10.17) concluding that there was a significant difference in ESR values on EDTA and citrate anticoagulants<sup>[12]</sup>. This might be because of the physical property of blood anticoagulated with EDTA which is slightly more viscous as compared to the blood

anticoagulated with TSC. This corresponds to the result reported from Tehran University, Iran, and implicated that there was a significant difference between the results of ESR tests which used two anticoagulants<sup>[19]</sup>. Besides this, in our study we found that the ESR values of males is bit high than those of female. This might be due to most of the patients are male of older age which contradict the result<sup>[20]</sup>.

## CONCLUSION

Present study showed ESR value of blood anticoagulated with EDTA is greater than that of blood anticoagulated with trisodium citrate with a mean difference of 8.03 mm/hour among patient with psychiatric illness. The t-value was 9.282 and the difference between both the anticoagulant for using ESR estimation is statistically significant. Different reference values may be incorporated for both the anticoagulant (EDTA and TSC) for ESR estimation for accurate report. Further studies are required to establish the study findings mainly in healthy individual and in persons with psychiatric illness from different parts of the world.

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