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Comparative Study of Erythrocyte Sedimentation Rate (ESR) three Sampling Techniques: Whole Blood in Tri-Sodium Citrate, Whole Blood in Ethylene Di Amine Tetra Acetic Acid (EDTA) , EDTA Blood Diluted with Tri–Sodium Citrate

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Authors' contributions

This work was carried out in collaboration among all authors. Author KHBPF conceptualized and designed the research, edited the research proposal through all stages, supervised data collection, edited and formatted manuscript to journal specifications upto final version. Author KSP supervised sample collection and testing. Author RSL guided statistical analysis. Authors HPAG, AWGMS wrote the project proposal, collected and analyzed data, wrote the project report. All authors read and approved the final manuscript.

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ABSTRACT

Background: ESR is a simple, inexpensive test commonly used for screening of infective, inflammatory and neoplastic processes. The recommended standard method for ESR by the International Committee for Standardization in Hematology(ICSH) is the Westergren method where

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blood is allowed to sediment under optimum conditions in a westergren tube for a given period of time Recommended samples to be used for the test by ICSH are citrate,EDTA diluted in citrate and direct EDTA. When direct EDTA is used ICSH gives a formula to calculate a value that corresponds to citrate values.

Aim: In our study we compared ESR values done on three different samples. Whole blood collected into 3.8% Trisodium-citrate(4:1)(Method1) , EDTA(Method 2), EDTA anticoagulated blood later diluted with 3.8% Tri sodium citrate(4:1)(Method 3)

Methodology: 194 patients' samples were analyzed irrespective of clinical conditions and gender. Samples were taken into citrate (in a ratio of 1:4, Citrate to blood) and EDTA anticoagulants. Citrate and EDTA samples were mounted directly into the westergren tubes and a third tube was mounted with EDTA blood from the EDTA tube diluted 1:4 with citrate in the laboratory.

Results: 194 results were grouped on HCT as 67 samples with HCT $\leq$0.35 and 127 with HCT > 0.35. Mean, SD and range of ESR for methods 1,2 and 3 were (33.81, 22.48,2-118) ,(46.53, 25.02 ,3-122) (32.31, 22.02,2-121)There was no significant difference between(*P*=0.23) method 1 and method 3. EDTA values were higher than citrated samples and difference was statistically significant (*P*=0.00). However when the values given by ICSH were substituted for EDTA values (method 2) they were comparable to methods 1 and 3(*P*=0.59; *P*=0.98) in patients with HCT<0.35. When HCT was >0.35 the difference was significant with the citrate samples (*P*=0.00) but not with the samples later diluted with citrate (*P*=0.103).All tests were conducted at 5% significance level.

Conclusion: ESR gives comparable values when samples are collected into citrate and when collected into EDTA and later diluted with citrate. When EDTA samples are directly mounted the corresponding value given by ICSH is valid when HCT is <0.35

Keywords: Erythrocyte Sedimentation Rate (ESR) on citrate and EDTA samples; Different samples for ESR; Citrate and EDTA anticoagulant for ESR.

1. INTRODUCTION

Erythrocyte sedimentation rate (ESR) is a simple, inexpensive, screening test commonly used in clinical practice which is determined by a complex interaction of various factors. The method for the ESR was first introduced by Polish Physician Edmand Biernacki in 1897 [1] and developed by Dr. A.Westergren and Dr. R.Fahraeus in 1921 [2].

ESR is widely used as a screening test to detect presence of an underlying infective, inflammatory or malignant process. The first expert International Council for Standardization of Haematology (ICSH) ESR panel was established in 1965 and its reference method was published in 1973[3]. Accordingly the reference method for measurement of ESR should be based on Westergren method. Samples to be used as recommended by ICSH are whole blood collected into EDTA and later diluted with sodium citrate or saline (4:1) and whole blood anticoagulated with sodium citrate [3]. In 1993, reference and standardized methods were proposed by ICSH, using undiluted EDTA blood samples. However the values obtained by this method did not give comparable results to values obtained on citrated samples. Therefore the ICSH derived a formula that can be used to convert EDTA results into comparable results.Accordingly Diluted blood ESR = (Undiluted blood ESR × 0.86) - 12 [4].

Several studies have been done by different investigators to compare the ESR technique.they have evaluated time of end point (30 Vs 60 minutes) verticality of the westergren tube (slanted Vs upright) and differences in anticoagulant of the sample.

A comparative study was conducted on ESR using Tri sodium citrate, Normal saline and whole blood in EDTA by Emelike et al in Nigeria in 2010. Blood specimens were collected from 200 apparently healthy individuals and ESR was compared between whole EDTA blood , EDTA diluted with citrate and EDTA diluted with normal saline. Setting ESR using whole blood showed a statistically significant difference, as the values obtained were higher than those obtained when samples were diluted with trisodium citrate and normal saline. [2]

Another comparative study of ESR using different anticoagulants, sodium citrate and EDTA was conducted by Shruti Kumta and others in India [5]. 22 Blood samples were collected from the OPD of Yenepoya University

hospital patients into two sets of blood collection vials containing EDTA and sodium citrate respectively. Values of ESR using EDTA were 4-6 mm lower than that of sodium citrate [5].

D. M. Dissanayaka , Faculty of Medicine, University of Peradeniya, Sri Lanka in 2006 has conducted a research regarding a rapid method for testing ESR. Blood samples from 153 patients were collected. One filled westergren tube was kept vertical and another tube was tilted 45 degrees from vertical. Quick and accurate results (in between 10.5 to 11.5 minutes) were given by the modified angle method [6].

In a study conducted by Getaneh et al. comparing ESR on EDTA and Citrate anticoagulated samples, [7] the mean difference of ESR values between the use of EDTA and TSC anticoagulated blood was 6.91 ± 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. In another study Salvagno et al observed that the percentage of samples with ESR >20 mm/h was significantly higher in K_2EDTA than in sodium citrate (66% *vs.* 47%; P=0.001) [8]. A study by Shallal et al on, Effect of different types of anticoagulants and storage period on the erythrocyte sedimentation rate in healthy and unhealthy people ,concluded that there was a decrease in ESR with citrate compared to EDTA and heparin, which was significant in both healthy and unhealthy people [9].

In 1988 the ICSH conducted a research to compare whole blood diluted with 3.8 % Tri sodium in 4:1 ratio (standardized method) with undiluted EDTA(reference method). On the results obtained they derived a formula to convert EDTA sample values to corresponding citrate values. They also recommended to use blood samples with haematocrit (HCT) less than 0.35 for conversion as higher HCT gave erroneous results. The formula derived was (Result of reference method × 0.86) - 12 = result of standardized method .A table for corresponding citrated and EDTA values was also drawn up.[4] .

Manual ESR testing is now being superseded by analyzing using automated analyzers. Recent studies have mostly been conducted to compare the various types of analyzers with the standard Westergren method. These studies have shown that auto analyzers give comparable results to the manual Westergren method [10-12].

Due to the varied methods in use, both manual and automated, the ICSH realizing the need for new recommendations to standardize reliable, reproducible comparable methods issued a new compilation in 2017. They elaborate that the gold standard is the standard Westergren method while acknowledging that there were many methods in use ranging 'from modest modifications of the Westergren method to very different methodologies'. They also concluded that 'Results obtained with the new instruments could differ from results obtained with the Westergren method by up to 142%' and that ' different non-Westergren methods showed differences from each other of up to 42%'. According to them the new methods 'often used standard EDTA tubes, eliminating the need for a dedicated ESR tube' [13].

ESR is used widely in paediatric practice together with the Full Blood Count and CRP for investigation of disease. FBC is usually carried out on an EDTA sample and ESR on a citrated sample. As pediatric sample collection is highly intensive and difficult to carry out, it is inconvenient to collect two samples for FBC and ESR from each patient. If Westergren method could be implemented to use EDTA sample for ESR one sample will be enough for both tests and it could be extremely convenient. In our study we aimed to compare ESR values done on three different samples, 1) direct EDTA 2) Citrate 3) EDTA sample later diluted in citrate.

By comparing the results we tried to ascertain if one EDTA sample would be adequate for FBC and ESR in both adults and children.

2. METHODOLOGY

2.1 Study Design

A cross sectional, analytical, descriptive study was carried out on patients who presented to the Out Patients Department and inward patients who were requested with ESR at the General Hospital ,Kalutara .

2.2 Inclusion Criteria

OPD and inward patients irrespective of the clinical condition who have been requested to have BOTH a Full blood count and ESR by the

clinicians. Patients who give informed consent to be enrolled in the study.

2.3 Exclusion Criteria

All pediatric patients under 2years of age.

2.4 Sample Size

Sample size was calculated by using the equation given below. It determines the minimum sample size to detect a specific difference in means and achieve desired values of type I and type II errors.

$$\Phi = \sqrt{n\sum_{i=1}^{a}\tau_i^2/a\sigma^2},$$

where $n =$ sample size, $a =$ number of groups, $\sigma^2 =$ pooled variance, $\tau_i = i$th treatment effect (mean of the ith group – overall mean).

Minimum sample size is determined based on the operating characteristic curve plots of β (probability of type II error) against the parameter Φ [14].

Based on a pilot study, group means ($\hat{\tau}_1 = -3.35, \hat{\tau}_2 = 12.2,$ and $\hat{\tau}_3 = -7.65$) and pooled variance ($\hat{\sigma}^2 = 23.69$) were calculated. At 5 % significant level, required minimum sample size with power (1-β) greater than 80% was 45. In current study, we were able to analyze 194 samples.

2.5 Sample Collection and Processing

Blood was collected from the median cubital vein with minimal haemostasis using sterile, disposable needles and syringes (bore size 23G for pediatric patients and 22G or for adults). 4 ml of blood was collected , 2 ml was added into tri sodium citrate anticoagulated tube and 3 ml into the EDTA tube (which will be also used for FBC) The test was carried out within 2 hours of sample collection by the Westergren method .A FBC was done from the EDTA sample to obtain the HCT . Thereafter, 3 ESR tests were carried out on each patient. One directly mounted from the citrate tube , one direct from the EDTA tube and the third from EDTA blood diluted in a 1:4 ratio with citrate . All tubes were kept undisturbed for one hour in vertical position and ESR recorded as millimeters per hour.

2.6 Statistical Analysis

Data were analyzed using statistical software Minitab 17. Mean ESR values with standard deviation (SD) and 95% confidence intervals were calculated for each blood sampling method. One way ANOVA was performed to compare mean ESR values in the above three methods. Tukey Simultaneous Test for differences of means was performed to assess the significance of difference between pair of groups. P value of < 0.05 was taken as statistically significant. Thus ESR results from following groups were analyzed to ascertain if there was a statistically significant difference between the means of the three methods,

1. Results obtained by analyzing the 194 samples - direct comparison of three groups of data
2. Total 194 results with substitution of ICSH recommended citrate value for EDTA result values compared with other two groups
3. Direct results obtained by analyzing 67 samples with ≤0.35 HCT - direct comparison of data
4. 67 samples (≤0.35 HCT) with substitution of ICSH recommended citrate value for EDTA result values with other two groups
5. Direct results obtained by analyzing 127 samples with >0.35 HCT – direct comparison of data
6. 127 samples (>0.35 HCT) with substitution of ICSH recommended citrate value for EDTA result values with other two groups

3. RESULTS AND ANALYSIS

In our study, we analyzed 194 patients' samples irrespective of clinical conditions and gender. Depending on haematocrit (HCT), we categorized the total of 194 results in to two groups as 67 samples with HCT ≤0.35 and 127 samples with HCT > 0.35. This was because ICSH in 1993 described a reference method to use undiluted EDTA whole blood for Westergren method ESR which used a formula , (Result of reference method × 0.86) - 12 = result of standardized method) to compare results between undiluted EDTA blood and blood diluted in citrate which showed correlation when the HCT was less than 0.35.

In our study, we followed ICSH recommended gold standard Westergren method and used

three combinations of anticoagulants and dilution factors. Each sample was tested in triplicate by these three techniques.

1. Whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1). (Method 1)
2. Undiluted whole blood anticoagulated with EDTA. (Method 2)
3. EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1). (Method 3)

First we analyzed direct results of all three methods in a head on comparison. Thereafter we substituted ICSH recommended values derived from the formula for EDTA results and analyzed those results with citrated and EDTA later diluted with citrate samples. As ICSH recommends that these results are valid only when HCT is < 0.35 we further analyzed this group as ICSH recommended values substituted for EDTA values in HCT < 0.35 group and HCT < 0.35 group. Test results therefore were analysed under the following groups,

1. Results obtained by analyzing the 194 samples - direct comparison of three groups of data
2. Total 194 results with substitution of ICSH recommended citrate value for EDTA result values compared with other two groups
3. Direct results obtained by analyzing 67 samples with ≤0.35 HCT - direct comparison of data
4. 67 samples (≤0.35 HCT) with substitution of ICSH recommended citrate value for EDTA result values with other two groups
5. Direct results obtained by analyzing 127 samples with >0.35 HCT – direct comparison of data
6. 127 samples (>0.35 HCT) with substitution of ICSH recommended citrate value for EDTA result values with other two groups

Each data group contains of three sets of data (method 1, 2 and 3). We used statistical software Minitab 17 for analysis of data.

3.1 Result

Samples collected into citrate and samples collected into EDTA and later diluted with citrate give similar test results but are different from ESR directly mounted from EDTA sample.

Total 194 results with substitution of ICSH recommended citrate value for EDTA result values
Method 1= X (Whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1).)
Method 2= Y (Undiluted whole blood anticoagulated with EDTA with substitution.)
Method 3= Z (EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1).)

Table 1. Mean, standard deviation and range of three methods

Method	Mean(mm/hr)	Standard Deviation	Range (mm/hr)
1. Whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1).	33.81	22.48	2- 118
2. Undiluted whole blood anticoagulated with EDTA.	46.53	25.02	3- 122
3. EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1).	32.31	22.02	2-121

Results obtained by analyzing the 194 samples

Comparison of means and standard deviations

Method 1= X (Whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1).)
Method 2= Y (Undiluted whole blood anticoagulated with EDTA.)
Method 3= Z (EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1).)

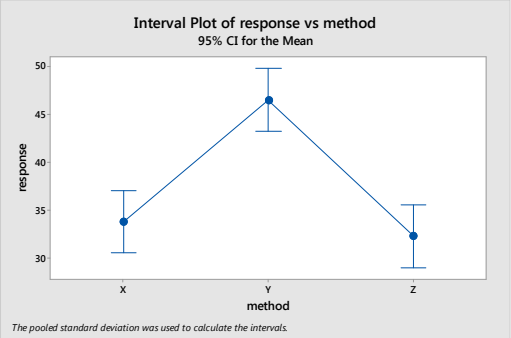

Fig. 1. Means and standard deviations of total 194 results in X, Y and Z methods

Method	N	Mean	StDev	95% CI
X	194	33.81	22.48	(30.54, 37.08)
Y	194	46.53	25.02	(43.26, 49.80)
Z				

Tukey Simultaneous Tests for Differences of Means

Difference Of method Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Ajusted P- Value
Y - X	12.722	0.913	(10.584, 14.860)	13.93	0.000
Z - X	-1.495	0.913	(-3.633, 0.643)	-1.64	0.230
Z - Y	-14.216	0.913	(-16.354, -12.079)	-15.56	0.000

Method	N	Mean	SD	95% CI
X	194	33.81	22.48	(30.75, 36.87)
Y	194	30.98	20.46	(27.93, 34.04)
Z	194	32.31	22.02	(29.26, 35.37)

Tukey Simultaneous Tests for Differences of Means

Difference Of method Levels	Difference of Means	SE of Difference	Simultaneous	T-Value	Adjusted P-Value
Y - X	-2.825	0.821	(-4.746, -0.903)	-3.44	0.002
Z - X	-1.495	0.821	(-3.416, 0.427)	-1.82	0.163
Z - Y	1.330	0.821	(-0.592, 3.251)	1.62	0.237

When ICSH recommended corresponding citrate values are used for the EDTA values they give comparable values to the EDTA samples and the samples collected into EDTA and later diluted with citrate but not the samples collected directly into citrate.

Direct results obtained by analyzing 67 samples with ≤0.35 HCT

Method 1= X (Whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1).)
Method 2= Y (Undiluted whole blood anticoagulated with EDTA.)
Method 3= Z (EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1).)

Method	N	Mean	StDev	95% CI
X	67	38.03	20.73	(32.74, 43.32)
Y	67	54.10	24.80	(48.81, 59.40)
Z	67	36.64	20.05	(31.35, 41.93)

Tukey Simultaneous Tests for Differences of Means

Difference of method Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
Y - X	16.07	1.55	(12.41, 19.74)	10.40	0.000
Z - X	-1.39	1.55	(-5.05, 2.27)	-0.90	0.643
Z - Y	-17.46	1.55	(-21.12, -13.80)	-11.30	0.000

Results of samples collected into EDTA vary significantly from test results of samples collected into citrate and samples collected into EDTA and later diluted with citrate when the subgroup of samples with HCT < 0.35 is analyzed as a separate group. The findings are similar to findings when the whole group of all 194 samples was analyzed.

67 samples (≤0.35 HCT) with substitution of ICSH recommended citrate value for EDTA result values

Method 1= X (Whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1).)
Method 2= Y (Undiluted whole blood anticoagulated with EDTA with substitution.)
Method 3= Z (EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1).)

Method	N	Mean	StDev	95% CI
X	67	38.03	20.73	(33.01, 43.05)
Y	67	36.88	21.70	(31.86, 41.90)
Z	67	36.64	20.05	(31.62, 41.66)

Tukey Simultaneous Tests for Differences of Means

Difference of method Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
Y - X	-1.15	1.42	(-4.51, 2.22)	-0.81	0.698
Z - X	-1.39	1.42	(-4.75, 1.98)	-0.98	0.593
Z - Y	-0.24	1.42	(-3.60, 3.13)	-0.17	0.985

When ICSH recommended corresponding citrate values are used for the EDTA values in the subgroup of samples with HCT < 0.35 they give comparable values to citrated samples. Therefore when HCT is < 0.35 EDTA values obtained can be used to find the corresponding citrate values by using the ICSH formula or table

Direct results obtained by analyzing 127 results with >0.35 HCT

Method 1= X (Whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1).)
Method 2= Y (Undiluted whole blood anticoagulated with EDTA.)
Method 3= Z (EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1).)

Method	N	Mean	StDev	95% CI
X	127	31.99	23.16	(27.91, 36.08)
Y	127	42.54	24.29	(38.45, 46.62)
Z	127	30.03	22.74	(25.95, 34.12)

Tukey Simultaneous Tests for Differences of Means

Difference Of method Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
Y - X	10.54	1.16	(7.82, 13.26)	9.07	0.000
Y - X	-1.96	1.16	(-4.68, 0.76)	9.07	0.210
Z - Y	-12.50	1.16	(-15.22, -9.78)	-10.76	0.000

Results of samples collected into EDTA vary significantly from test results of samples collected into citrate and samples collected into EDTA and later diluted with citrate when the subgroup of samples with HCT > 0.35 is analyzed as a separate group. The findings are similar to findings when the whole group of all 194 samples was analyzed.

127 results (>0.35 HCT) results with substitution of ICSH recommended citrate value for EDTA result values

Method 1= X (Whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1).)
Method 2= Y (Undiluted whole blood anticoagulated with EDTA with substitution.)
Method 3= Z (EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1).)

Method	N	Mean	StDev	95% CI
X	127	31.99	23.16	(28.20, 35.79)
Y	127	27.90	19.12	(24.10, 31.69)
Z	127	27.90	22.74	(26.24, 33.83)

Tukey Simultaneous Tests for Differences of Means

Difference Of method Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
Y - X	-4.09	1.05	(-6.54, -1.65)	-3.92	0.000
Z - X	-1.96	1.05	(-4.41, 0.49)	-1.87	0.146
Z - Y	2.13	1.05	(-0.31, 4.58)	2.04	0.103

When ICSH recommended corresponding citrate values are used for the EDTA values in the subgroup of samples with HCT > 0.35 they give significantly different results with the citrate samples but not with the samples later diluted with citrate. As the gold standard is the samples directly collected into citrate EDTA substitution values are not accurate when the HCT of the sample is > 0.35.

3.2 Summary of results and analysis

Table 2. Summary of results and analysis

Analyzed group	Results (P value obtained) **P < 0.005 = significant** **P > 0.005 = not significant**	Result interpretation
1). Results obtained by analyzing the 194 samples	Y-X = 0.000 Z-X = 0.230 Z-Y = 0.000	Samples collected into citrate and samples collected into EDTA and later diluted with citrate give similar test results.But samples collected into EDTA give a significantly different result to the citrated samples.

2). Total 194 results with substitution of ICSH recommended citrate value for EDTA result values	Y-X = 0.002 Z-X = 0.163 Z-Y = 0.237	When ICSH recommended corresponding citrate values are used for the EDTA values they give comparable values to the EDTA samples and the samples collected into EDTA and later diluted with citrate but not the samples collected directly into citrate.
3). Direct results obtained by analyzing 67 samples with ≤0.35 HCT	Y-X = 0.000 Z-X = 0.643 Z-Y = 0.000	Results of samples collected into EDTA vary significantly from test results of samples collected into citrate and samples collected into EDTA and later diluted with citrate when the subgroup of samples with HCT < 0.35 is analyzed as a separate group. The findings are similar to findings when the whole group of all 194 samples was analyzed.
4). 67 samples (≤0.35 HCT) with substitution of ICSH recommended citrate value for EDTA result values	Y-X = 0.698 Z-X = 0.593 Z-Y = 0.985	When ICSH recommended corresponding citrate values are used for the EDTA values in the subgroup of samples with HCT < 0.35 they give comparable values to citrated samples. Therefore when HCT is < 0.35 EDTA values obtained can be used to find the correseponding citrate values by using the ICSH formula or table.
5). Direct results obtained by analyzing 127 results with >0.35 HCT	Y-X = 0.000 Z-X = 0.210 Z-Y = 0.000	Results of samples collected into EDTA vary significantly from test results of samples collected into citrate and samples collected into EDTA and later diluted with citrate when the subgroup of samples with HCT > 0.35 is analyzed as a separate group. The findings are similar to findings when the whole group of all 194 samples was analyzed.
6). 127 samples (>0.35 HCT) with substitution of ICSH recommended citrate value for EDTA result values	Y-X = 0.000 Z-X = 0.146 Z-Y = 0.103	When ICSH recommended corresponding citrate values are used for the EDTA values in the subgroup of samples with HCT > 0.35 they give significantly different results with the citrate samples but not with the samples later diluted with citrate. As the gold standard is the samples directly collected into citrate EDTA substitution values are not accurate when the HCT of the sample is > 0.35.

X = Mean of the results obtained for ESR by whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1). Y = Mean of the results obtained for ESR by undiluted whole blood anticoagulated with EDTA. (in groups 2,4 and 6 Y= Mean of the results obtained for ESR by undiluted whole blood anticoagulated with DTA with substitution)Z= Mean of the results obtained for ESR by EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1)

4. DISCUSSION

ESR is a commonly used clinical test useful for screening for infective , inflammatory and neoplastic conditions. It is done by the Westergren method in which a sample of anticoagulated blood is allowed to sediment over a given period of time in a standardized Westergren tube under optimum conditions. [15] Recently new methods mostly deviations of the westergren method and alternate methods have been introduced worldwide [13]. Many automated analyzers which analyze ESR have also been introduced and are widely used [13]. Several studies have been carried out to ascertain the comparability of these analyzers with the Westergren method [10-12]. However the ISCH recognized that there can be marked differences in the test results with these methods and still identifies the Westergren method as the 'Gold standard' [13].

Several anticoagulants have been in used for sample collection for the Westergern method. According to the ICSH recommendation both whole blood directly anticoagulated with 3.8% Tri sodium citrate 4:1 and pre EDTA anticoagulated samples diluted with 3.8% Tri sodium citrate 4:1 can be used for the standardized Westergren method [4][13].

Due to the hazardous nature of handling of potentially infectious blood when diluting EDTA anticoagulated blood with citrate, ICSH accepted direct mounting from EDTA anticoagulant [4]. Since EDTA results did not give comparable results ICSH devised a formula and set of values to be substituted to EDTA results.

In our study, we strived to compare ESR values of 194 patients done on three different samples. Whole blood collected into the anticoagulant 3.8% Tri sodium citrate (4:1) (Method 1), Undiluted whole blood anticoagulated with EDTA (Method 2), EDTA anticoagulated blood diluted with 3.8% Tri sodium citrate (4:1) (Method 3).

Although ESR results of samples collected into EDTA and subsequently diluted in citrate were slightly lower than whole blood directly anticoagulated with 3.8% Tri sodium citrate 4:1 there was no significant difference (P value 0.230) between values obtained on the two samples. Results of our study are compatible with the ICSH recommendation that blood collected into citrate and blood collected into EDTA and later diluted (1:4) in citrate give comparable results . Even when the samples were grouped according to HCT < or > 0.35 the difference was not significant between these two sampling methods. (p=0.643 and p=0.210) There are no other studies in literature found comparing blood collected into citrate with blood collected into EDTA and later diluted with citrate.

However blood collected into EDTA and directly mounted gave statistically different results to the other two methods when the group was analyzed as total (n=194 , p= 0.000 and p=0.000) and in the group of HCT < 0.35 (n= 67, p=0.000 , p= 0.000) and group of HCT > 0.35 (n= 127, p=0.000 and p=0.000). This was also seen in a study by Getaneh et al who compared ESR on EDTA and Citrate samples [7]. In their study the mean difference of ESR values between the use of EDTA and TSC anticoagulated blood was 6.91 ± 13.66 mm/h with a t-value of 4.24 (P < 0.0001; 95% CI 3.66–10.17). In another study by Salvagno et al observed that the percentage of samples with ESR >20 mm/h was significantly higher in K_2ED TA than in sodium citrate (66% *vs.* 47%; P=0.001) [8]. A study by Shallal et al on, 'Effect of different types of anticoagulants and storage period on the erythrocyte sedimentation rate in healthy and unhealthy people' , also concluded that there was a decrease in ESR with citrate compared to EDTA and heparin, which was significant in both healthy and unhealthy people [9]. Thus our study shows comparable results to other studies in that EDTA samples give statistically different results to citrated samples.

In 1993, ICSH recommended to use undiluted EDTA anticoagulated whole blood with HCT ≤ 0.35 as a reference method by substituting given corresponding values [4]. In our study although direct readings of this method (reference method) were higher than the standard routine methods ,when substituting the ICSH recommended corresponding value for EDTA values in patients with HCT≤ 0.35 (67 samples), there was no significant difference (P value 0.698 and P value 0.985 when compared to citrated samples and samples collected to EDTA and later diluted with citrate. Thus ESR performed directly with blood collected into EDTA and substituted with the corresponding ESR value given by ISCH (Annexure 1) gives comparable results in all 3 methods when the HCT is < 0.35 .

In the 127 samples with HCT > 0.35 EDTA value substitution gives significantly different results

with the citrate samples (P= 0.00) but not with the samples later diluted with citrate (0.103) .Thus ICSH substituted values for EDTA samples do not give comparable results to citrated samples when HCT > 0.35. There are no studies in literature comparing substitution of EDTA comparison values with citrate samples but our findings confirm the validity of the recommended values for EDTA made by ICSH.

According to the results and analysis, it was observed that compatibility values for reference method were over compensating the corresponding values. Observing the scattering of data to fit with method 1 and method 3, we proposed new compatibility values for the reference method. Thus a new proposed set of values have been documented by this study for ESR results obtained by EDTA samples directly hen HCT >0.35. (annexure 2) However further studies involving normal subjects and a larger number of participants needs to be carried out to determine and validate a reference range for EDTA sample values when HCT > 0.35. This can be further described according to gender and age limits.

4. CONCLUSION

Blood collected directly into citrate anticoagulant and blood collected into EDTA and later diluted with citrate give comparable ESR results irrespective of patients HCT. Therefore whenever possible these samples should be used for ESR testing. Direct mounting of ESR from the EDTA samples gives statistically different results to the citrated samples. When the ICSH recommended values are substituted for EDTA values they give comparable results when the HCT < 0.35. Therefore when a single sample is available for FBC and ESR in an EDTA anticoagulated tube , specially in pediatric patients it can be used by diluting with citrate or direct mounting and correction of values according to ICSH recommendation if HCT is <0.35. When HCT is above >0.35 substitution of ICSH values did not give corresponding results. We have established a corresponding set of values for EDTA direct mounted samples when HCT > 0.35 but recommend further studies with larger numbers for validation of these results.

ETHICAL APPROVAL

Ethical clearance was obtained from the Ethical Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura (Ref No: MLS 05/2017) Permission was taken from the Director of General Hospital, Kalutara. As the study was done on hospitalized patients who have been requested both ESR and FBC by clinicians there was no additional pricking or additional drawing of blood specially intended for this research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Grzybowki A, Sak JJ. Who Discovered the Erythrocyte Sedimentation Rate? J Rheumatol [Internet]. 2011;38(7):1521–2. Available:http://www.jrheum.org/cgi/doi/10.3899/jrheum.101312
2. Emelike OF, Akpan JE, Obigwe BU, Jeremiah Z. Comparative Study of Erythrocyte Sedimentation Rate (ESR) Using Trisodium Citrate , Normal Saline and Whole Blood in Ethylene Di Amine Tetra Acetic Acid (EDTA). J Appl Sci Environ Manag. 2010;14(1):23–7
3. Jou JM, Lewis SM, Briggs C, Lee SH, De La Salle B, Mcfadden S. ICSH review of the measurement of the erythocyte sedimentation rate. Int J Lab Hematol. 2011;33(2):125–32.
4. ICSH recommendations for measurement of erythrocyte sedimentation rate. International Council for Standardization in Haematology (Expert Panel on Blood Rheology) [published correction appears in J Clin Pathol 1993 May;46(5):488]. *J Clin Pathol*. 1993;46(3):198-203. DOI:10.1136/jcp.46.3.198
5. Kumta S, Nayak G, Shantaram M, Communication S. A comparative study of erythrocyte sedimentation rate (esr) using sodium citrate and edta 1 2. Int J Pharm Biol Sci. 2011;1(4):1–4.
6. Dissanayake D. A rapid method for testing

the erythrocyte sedimentation rate. J Diagnostic Pathol [Internet]. 2011;5(1):47–51.
Available:http://www.sljol.info/index.php/JDP/article/view/2960

7. Getaneh Z, Ayelgn F, Asemahegn G. et al. A comparison of erythrocyte sedimentation rates of bloods anticoagulated with trisodium citrate and EDTA among TB presumptive patients at the University of Gondar comprehensive specialized hospital, northwest Ethiopia. BMC Res Notes. 2020;13:113 Available: https://doi.org/10.1186/s13104-020-04963-0
8. Salvagno GL, Dima F, Lippi G. Nonequivalence of erythrocyte sedimentation rate assessed in whole blood anticoagulated with K 2 EDTA or sodium citrate.J Lab Precis Med. 2020; 5-12
9. Shallal AF, Ibrahim ZH, Kheder R, Hussein SH, Hassan SM, Khalil OA. Effect of different types of anticoagulants and storage period on the erythrocyte sedimentation rate in healthy and unhealthy people.Biomedical Research. 2020;31(1):9-12
10. Bogdaycioglu N, Yilmaz FM, Sezer S, Oguz E. Comparison of iSED and Ves-Matic Cube 200 erythrocyte sedimentation rate measurements with Westergren method. J Clin Lab Anal. 2015;29:397- 404.
11. Van Der Maas A, van den Ende CH, van Eerd J, Fransen J, den Broeder AA. The use of different methods for rapid determination of the ESR induces DAS28 misclassification in clinical practice. Clin Exp Rheumatol. 2010;28:477- 482.
12. Horsti J, Rontu R, Collings A. A comparison between the StaRRsed auto-compact erythrocyte sedimentation rate instrument and the Westergren method. J Clin Med Res. 2010;2:261- 265
13. Kratz A, Plebani M, Peng M, Lee YK, McCafferty R, Machin SJ; International Council for Standardization in Haematology (ICSH). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. Int J Lab Hematol. 2017;39(5):448-457.
DOI: 10.1111/ijlh.12693. Epub 2017 May 12.
PMID: 28497537.
14. Montgomery DC. Design and analysis of experiments. New York: John Wiley; 2001.
15. Lewis SM, Bain BJ, Bates I. Dacie and Lewis Practical Haematology [Internet]. 10th Ed Churchill LivingstonElsevier. 2006; 722 .
Available:http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Dacie+and+Lewis+Practical+Haematology,+10th+ed#0

Annexure 1. ESR values (mm) for verification of comparability of working (routine) method with ICSH standardized (undiluted EDTA) method [Ref : 4]

Undiluted EDTA value	corresponding Citrated (1:4) value	Undiluted EDTA value	corresponding Citrated (1:4) value	Undiluted EDTA value	corresponding Citrated (1:4) value
15	3-13	45	18-37	75	40-68
16	4-14	46	18-38	76	40-69
17	4-15	47	19-38	77	41-70
18	4-15	48	20-39	78	42-71
19	5-16	49	20-40	79	43-72
20	5-17	50	21-41	80	44-73
21	6-17	51	22-42	81	45-74
22	6-18	52	22-43	82	45-76
23	6-19	53	23-44	83	46-77
24	7-19	54	24-45	84	47-78
25	7-20	55	24-46	85	48-79
26	8-21	56	25-47	86	49-80
27	8-21	57	26-48	87	50-82
28	9-22	58	26-49	88	51-83
29	9-23	59	27-50	89	52-84
30	10-24	60	28-51	90	53-86
31	10-25	61	29-52	91	54-88
32	11-25	62	29-53	92	55-89
33	11-26	63	30-54	93	56-90
34	12-27	64	31-56	94	57-91
35	12-28	65	32-57	95	58-93
36	13-29	66	32-58	96	59-94
37	13-30	67	33-59	97	59-94
38	14-30	68	34-60	98	60-95
39	14-31	69	35-61	99	61-96
40	15-32	70	35-62	100	62-98
41	15-33	71	36-63	101	63-99
42	16-34	72	37-64	102	64-100
43	17-35	73	38-65	103	65-101
44	17-36	74	39-66	104	66-103
				105	67-104

Annexure 2. Proposed new compatibility values for reference method when HCT> 0.35

Undiluted EDTA Value (mm)	Propose Citrate value (mm)	Median to earest mm	Undiluted EDTAValue (mm)	Propose Citrate value (mm)	Median to nearest mm
15	3-13	8	34	13-28	21
16	4-14	9	35	13-29	21
17	4-15	10	36	14-30	22
18	4-15	10	37	14-31	23
19	5-16	11	38	15-31	23
20	5-17	11	39	15-32	24
21	6-17	12	40	16-33	25
22	6-18	12	41	16-34	25
23	6-19	13	42	17-35	26
24	7-19	13	43	18-36	27
25	7-20	14	44	18-37	28
26	8-21	15	45	19-38	29
27	8-21	15	46	19-39	29
28	9-22	16	47	20-39	30
29	9-23	16	48	21-40	31
30	10-24	17	49	21-41	31
31	10-25	18	50	22-42	32
32	12-26	19	51	23-43	33
33	12-27	20	52	23-44	34
53	24-45	35	82	48-79	64
54	25-46	36	83	49-80	65
55	25-47	36	84	50-81	66
56	27-49	38	85	51-82	67
57	28-50	39	86	52-83	68
63	32-56	44	87	53-85	69
64	33-58	46	88	54-86	70
65	34-59	47	89	55-87	71
66	34-60	47	90	56-88	72
67	35-61	48	91	56-89	73
68	36-62	49	92	57-91	74
69	37-63	50	93	58-92	75
70	37-64	51	94	59-93	76
71	38-65	52	95	60-94	77
72	39-66	53	96	61-96	79
73	40-67	54	97	62-97	80
74	41-68	55	98	63-98	81
75	42-70	56	99	64-99	82
76	42-71	57	100	65-101	83
77	43-72	58	101	66-102	84
78	44-73	59	102	67-103	85
79	45-74	60	103	68-104	86
80	46-75	61	104	70-105	88
81	48-77	63	105	72-105	89

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