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Comparison of erythrocyte sedimentation rate by Alifax Roller 20 LC method and standard Westergreen method

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Abstract
Erythrocyte sedimentation rate (ESR) is the most common test done for diagnosis and follow up treatment in patients suffering from autoimmune disorders and chronic illness. It is imperative for clinicians to understand the methodology, limitations and variations in testing platforms for ESR from ICSH standardized Westergreen method to various automated testing techniques. We conducted a retrospective study of 150 patients who were tested for ESR using Westergreen method and new automated Alifax Roller 20LC. The data was analyzed by applying Pearsons coefficient of correlation and Bland Altman analysis. It was observed that the automated Alifax Roller 20LC system tends to estimate ESR with positive bias. The maximum agreement between two methods was observed for ESR values between 20-40 mm/hr. As the likelihood of two similar results between two methods is uncertain, the difference between values should be acceptable clinically before replacing the ICSH Westergreen method with any new automated system.

Keywords: ESR, Westergreen method, roller LC, bland Altman analysis

Introduction
Erythrocyte sedimentation rate (ESR) was earliest described by Dr. R Fahraeus and Dr. A Westergreen in 1921 [1, 2]. ESR finds its application as a screening test for chronic illness and inflammation [3]. ESR is non specific as it increases in wide range of pathologies such as infections, autoimmune disease and malignancy. The test for ESR is influenced by red cell shape, size, Hematocrit and by other confounding factors [4]. The process of ESR comprises of three stages: aggregation, precipitation and packing. The most important stage is aggregation which is influenced by high molecular weight component of the plasma and Red blood cell structure [5]. The traditional ICSH Westergreen method based on sedimentation property is influenced by Hematocrit, temperature and vibrations. Although it remains gold standard but problems with dilution, mixing of blood samples, lack of controls & calibrators and pipettes make it a technically demanding test [6]. The newer tests based on capillary photometry are free of all shortcomings of the Westergreen method like Hematocrit influence, setup, mixing etc. and they are much more quick and cheaper [7].
Dr. A Westergreen was the foundation member of first expert International committee of standardization in hematology (ICSH) ESR panel in 1965. The ICSH revision for ESR methodology was done in 1973, 1977, 1988 and 1993 [8, 9, 10]. The ICSH-1993 document for ESR recommended the use of non diluted EDTA sample with packed cell volume (PCV) of 0.35 or less for performing reference method. The standardized method described was similar to the reference method except that it could use glass and plastic pipettes. The results of reference method [11] and ESR result had to be expressed as for diluted blood at 60 minute or normalized to 60 minutes, with the result expressed as ESR= x mm.
Routine Westergreen method (diluted) = (undiluted Westergreen method X 0.86)-12.

Study Design
We conducted a retrospective study in the department of Pathology & Transfusion Medicine, in a tertiary care hospital in western India. The study consist of 150 cases of ESR estimation including patients of all ages, male and female in a month's time period who had Hematocrit less than 35%. We use Alifax Roller 20LC (Italy) capillary photometry for ESR estimation in our setup. The department has a policy for samples with low Hematocrit less than 35% to perform a manual Westergreen method for ESR estimation, reporting is done by using the

correction formulae for manual ESR. Retrospective data of one month was collected where ESR values were reported based on Westergreen method for low Hematocrit values. The manual values were compared with their corresponding automated Roller 20LC results. The automated method and manual method were performed by two different technicians and two sides were blinded to the results of alternate technique. The data was analyzed using online free statistical software (Minitab). The comparison of both methods was done using Pearson's coefficient of correlation estimation and Bland Altman plot.

Results

The study population consists of 150 cases comprising of 73 (48.67%) males and 77 (51.33%) females. (Table-1) The minimum age of patient in our study was 2 years and maximum being 81 years. The largest proportion comprised of patients > 50 years of age about 49 (32.7%), followed by 40-50 year age group 27 (18.0%), 26 (17.3%) in 30-40 year age, 19 (12.7%) in 20-30 year age group, 14 (9.3%) and 15 (10%) in 10-20 year age group & 0-10 years of age. (Table-2) Most of the patients had Hematocrit values between 25-30, 45(30.0%). None of the patients had Hematocrit value of less than 5% in our study.(TABLE-3) The statistical analysis calculated Pearson's coefficient of correlation between two methods which was R= 0.7021 (95% confidence interval, Cl = 0.3168- 0.9478, p<0.001) and the regression equation was y=5.853 + 0.406 x. (Fig-1) Notwithstanding a determination coefficient of 0.6934, the differences between the two measurements can be seen better in Bland Altman plot that defined a bias of -24.42 units and an agreement range from -27.41 and 68.26 units. (Table-4)

Discussion

ESR is a popular test to perform and provides information to the physician in a quick manner [12]. Now a day traditional Westergreen method is generally not used in routine laboratories. It is important that while making comparison between Alifax Roller 20LC and standardized method the nature of sample- diluted or undiluted is kept in mind. As most of the automated analyzer use undiluted sample whereas standardized methods can use either diluted or undiluted samples, while comparing two results sample harmony and matrix has to be similar else the conclusion will be invalid [13, 14, 15]. The newer technologies tend to use undiluted EDTA samples for making them user friendly without the need of transferring samples from EDTA vials to ESR vial for making them user friendly and economical. ICSH-1993 guideline recommends that only those systems that only those systems that give results as Westergreen method with diluted blood at 60 minutes or normalized to 60 minutes have clinical value.

There were misleading interpretations of the reference method and lot of confusion existed about the use of standardized method. ICSH expert panel in 2010, has established changes of the recommendations for reference method and eliminated standardized method. The terminology ESR is retained although ICSH recognizes that single measurement after 60 minutes is not a rate [16].

Under mentioned principles should be borne in mind while performing ESR [16]:

- Blood collection by clean veni puncture over maximum period of 30 seconds is desirable.
- Manual or vacuum extraction can be done and blood should be collected in EDTA (K3 or K2) anticoagulant or sterile trisodium citrate dehydrates.
- The EDTA sample should be diluted with sodium citrate in portion of 4 volumes of blood to 1 volume of citrate.
- Blood citrate can be stored for 2 hours at ambient temperature or for 4 hours at 4C0 prior to testing.
- Standardized blood volume collection tubes should have minimum of eight complete inversions with air bubble travelling from end to end of the tube. It should be carried out until immediately before filling of ESR pipette.
- ESR pipette must be disposable but under special situations glass pipette can be re used after proper washing & drying. Pipettes should be colorless, circular, of sufficient length with diameter <2,55mm and constant bore (5% variation) throughout the tube. Pipettes should be filled with anticoagulant blood to a level at least 200 mm. They should be held vertical and protected from vibrations with maintenance of temperature (+/- 10C) within range of 18 ^{0}C– 25 ^{0}C.
- The results are to be recorded as sedimentation occurring at 60 minutes from the beginning of the test and expressed as ESR= x mm.

The Alifax Roller 20LC analyzer measures sedimentation and aggregation by optical density and ESR by an infrared micro photometer (950 nm) [17]. The blood is distributed in capillary and moves by hydrodynamics. The aggregation & sedimentation is read at 1000 times in 20 seconds, the electronic signals measured during rotation are directly related to number of RBC in capillary section at that time that are read by receiving photodiode. The impulse measured per unit time is a measure of ESR. The mean decrease in the signal per unit of time and square root of 'integral signal' are transformed to comparable Westergreen value [18].

In the present study, results obtained from Alifax Roller 20LC automatic ESR analyzer were compared with Westergreen method following correction for low Hematocrit using the agreement analysis of Bland & Altman [19]. Bland & Altman not only asses agreement & mean difference between the two methods but also asses the limits of agreement. By calculating standard deviation of the differences (+/-1.96), the two methods can be used interchangeably only when they do not affect clinical interpretation.

The Bland Altman analysis of various categories according to Hematocrit was done with calculation of mean of difference, standard deviation and upper & lower limits respectively. (Table-4) Regression analysis of two methods showed a correlation coefficient r=0.7021. The two methods have a positive correlation but to ascertain the agreement we did a Bland Altman analysis. In samples with Hematocrit values less than 35, corrective formulae by Fabry T L for Hematocrit was used [20].

Corrected ESR= ESR x 15/ 55-hematocrit

The Hematocrit wise Bland Altman analysis for manual and Alifax Roller 20LC automated method for one hour is

shown in Figures-2-9. These graphs between difference & mean by two methods did not reveal significant results that could be reliably interpreted due to small sample size although the values were within acceptable limits. (Fig-2-9) The Bland Altman analysis of all samples (Fig-9) shows a mean difference of 20.42 with 68.265 upper & -27.411 lower limits (as 95% limits of agreement). The following observations were made:

- Most of the values are within acceptable limits of agreement.
- The Alifax Roller 20LC system tends to measure higher ESR value in comparison to standard Westergreen method. (Table- 4&5) As indicated by positive mean of difference values for each categories.
- For low ESR values (ie <20) the Westergreen values tend to be slightly higher than Alifax Roller 20LC values ie the Roller LC has a negative bias.
- For ESR values between 20-40 both the methods are in agreement.
- For ESR values > 40- Alifax Roller 20LC shows positive bias ie higher than Westergreen values.
- For high ESR values > 60- Roller LC tends to measure ESR with positive bias and few values were beyond the limits of agreement (+/- 1.96 SD)
- Funneling effect was observed on Bland Altman analysis suggesting that there was discrepancy between two methods for high ESR values. It implies that for higher ESR values the values obtained by two methods were correlated but not agreement. The Alifax Roller 20LC overestimated ESR values when they were on higher side, which is clinically unacceptable.

On doing literature search, we found that such discrepancies between automated ESR estimation methods and standard Westergreen methods are reported in literature. Our findings are similar to Alfadhli *et al*. [21] and Caswellet *et al*. [22] who also obtained low agreement between manual and automated ESR estimation methods in their study. The results obtained with two ESR estimation methods showed good correlation when analyzed by simple Pearson's regression analysis on the other hand the Bland Altman analysis showed poor agreement on higher values, these findings are similar to those by Plebani M *et al*. [23]

Table 1: Sex wise distribution

Sex	No of patients
Male	73 (48.67%)
Female	77 (51.33%)
Total	150(100)

Table 2: Age wise distribution

Age	No of patients
0-10	15 (10%)
10-20	14 (9.3%)
20-30	19 (12.7%)
30-40	26 (17.3%)
40-50	27(18.0%)
>,=50	49 (32.7%)
Total	150(100)

Table 3: Hematocrit wise distribution

Hematocrit	No of patients
0-5	0
5-10	8 (5.3%)
10-15	12 (8.0%)
15-20	13 (8.7%)
20-25	25 (16.7%)
25-30	45 (30.0%)
30-35	34 (22.7%)
>35	13(8.6%)
Total	150(100)

Table 4: Simple mean SD for various Hematocrit categories

Category	N	Mean	SD	Upper limit	Lower limit
A	0				
B	8	13.13	29.162	70.28	-44.03
C	12	9.5	13.85	36.65	-17.65
D	13	36.31	40.14	114.98	-42.37
E	25	20.64	21.43	62.64	-20.57
F	45	23.38	26.93	76.16	-29.4
G	37	20.7	16.69	52.71	-12.71
H	10	3.9	3.93	11.6	-3.8
ALL	150	20.4267	24.40743	68.26526	-27.41186

Table 5: Simple mean, SD for three groups based on ESR

Category	N	Mean	SD	Upper limit	Lower limit
ESR < 20	84	16.99	21.64	60.27	26.29
ESR 20-40	40	26.65	27.39	54.78	81.43
28.13ESR >40	26	21.96	26.86	75.68	31.76

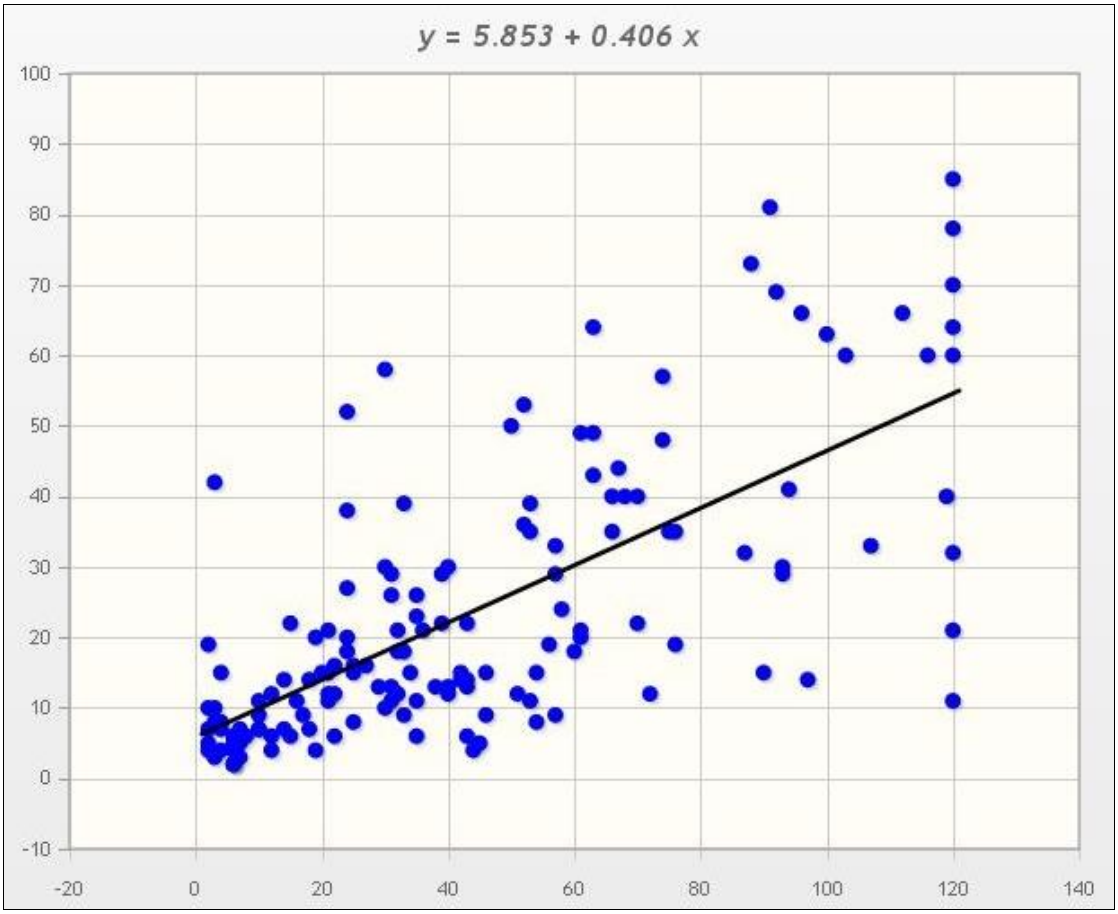

Fig 1: Regression analysis curve

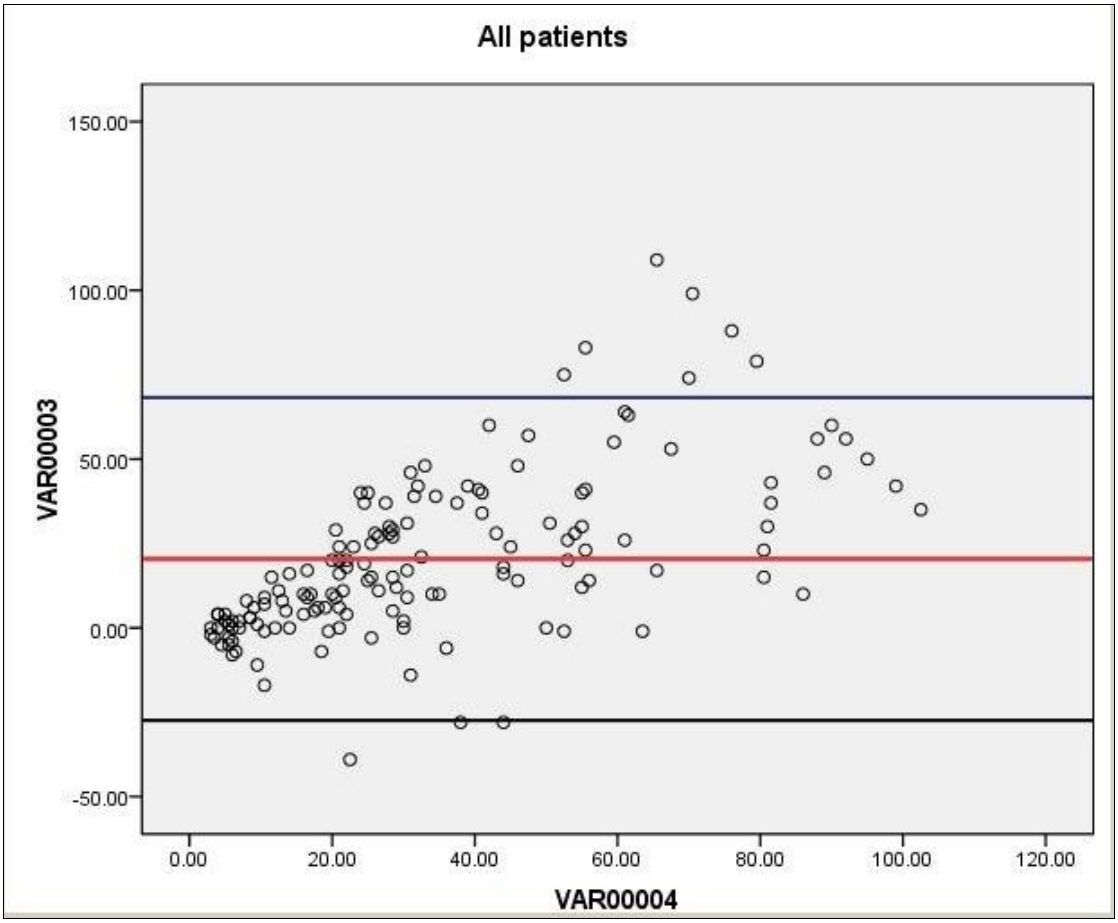

Fig 2: Bland Altman analysis (All patients)

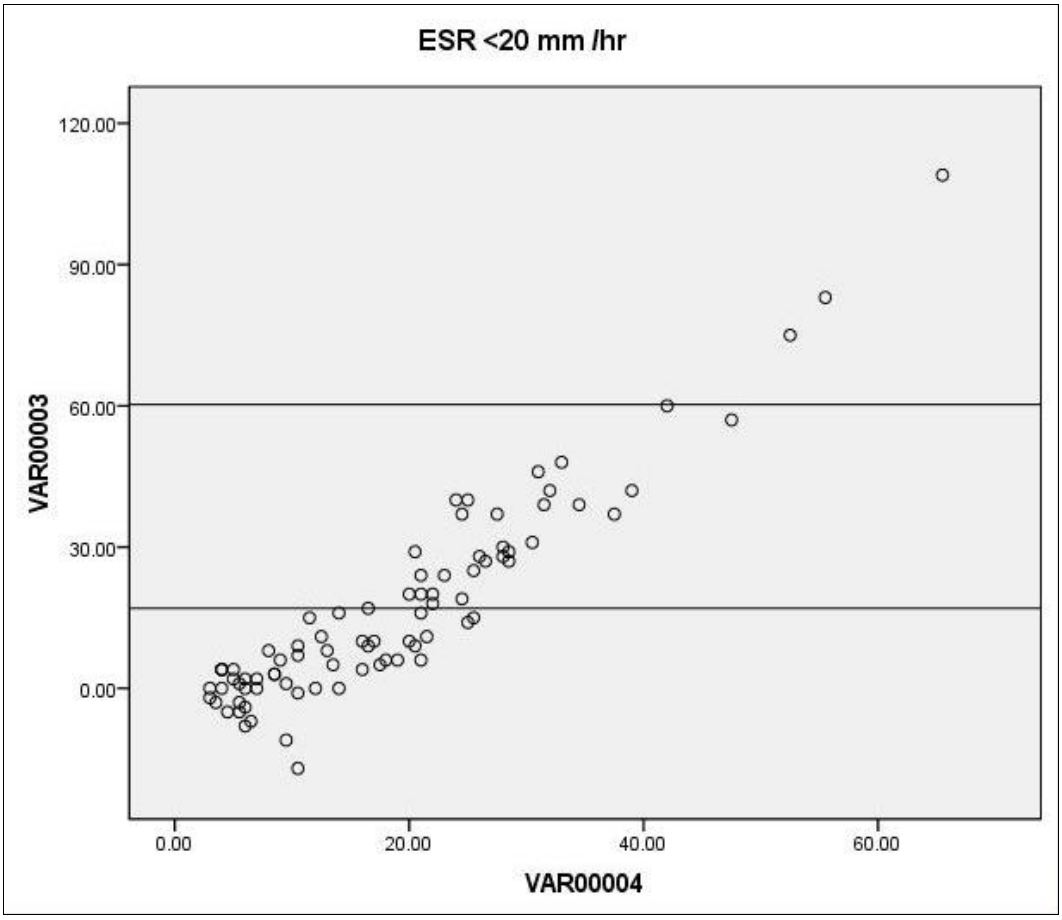

Fig 3: Bland Altman analysis (Patients with ESR < 20 mm per hr)

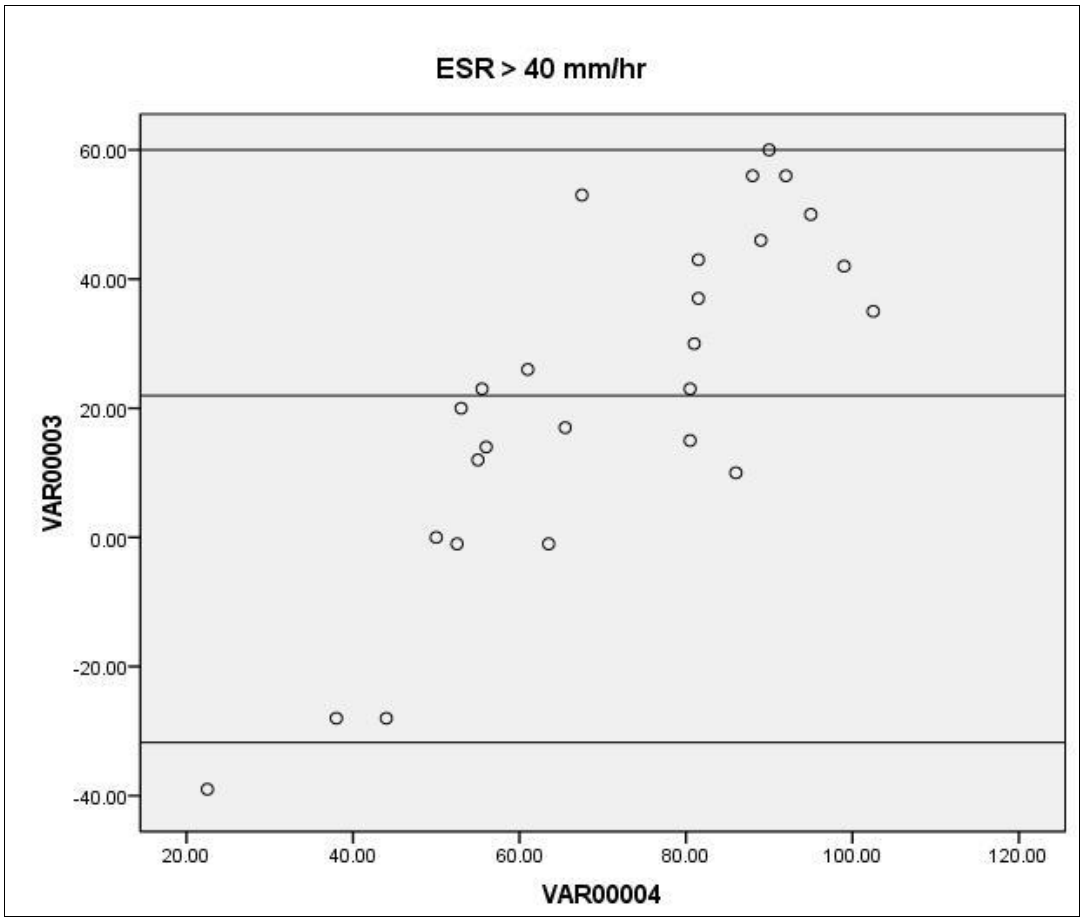

Fig 4: Bland Altman analysis (Patients with ESR > 40 mm per hr)

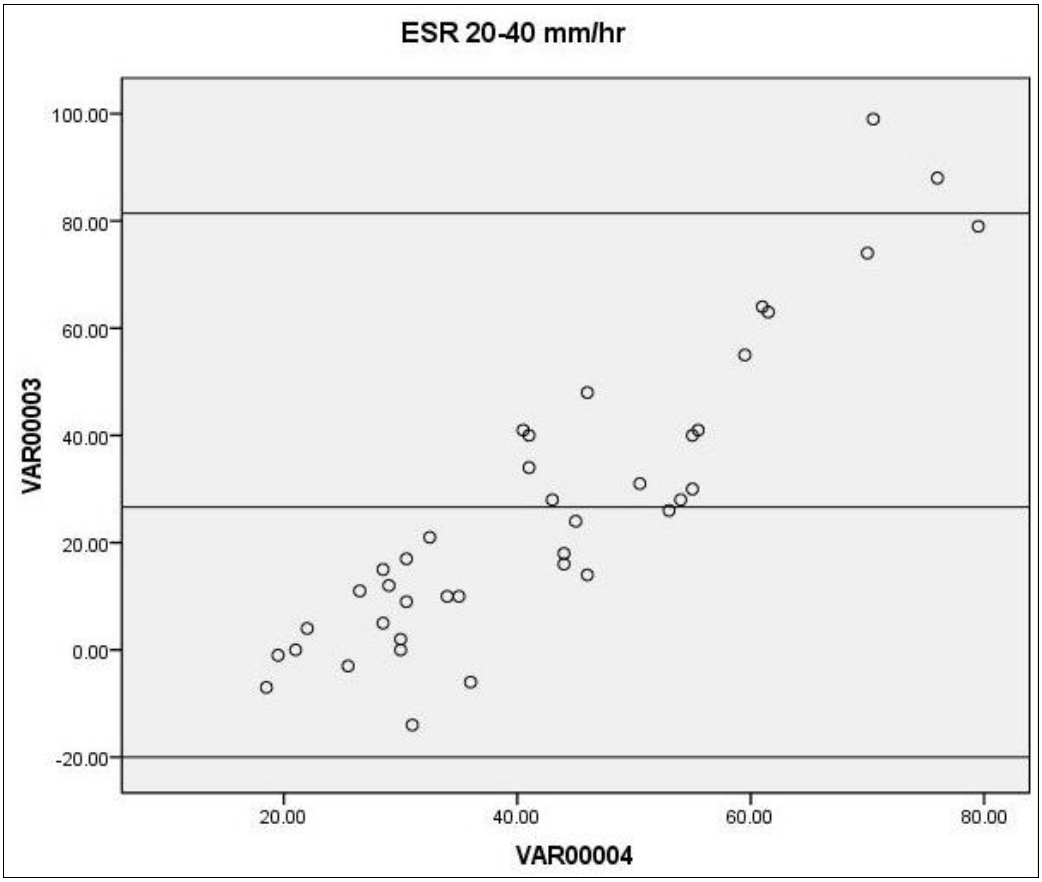

Fig 5: Bland Altman analysis (Patients with ESR 20-40 mm per hr)

Conclusion

It is uncertain to obtain similar results by two methods hence the difference between the two methods should be clinically acceptable then only the new method can be justified in replacing the ICSH standardized Westergreen method for ESR estimation.

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