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A Comparative Evaluation of Macro and Micro Methods for Determination of ESR and PCV in Health and Disease

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ABSTRACT

Background: The erythrocyte sedimentation rate is the rate at which erythrocytes settle down when anticoagulated blood is allowed to stand in a vertical column for a particular period (usually during 1st. hour) and the length of fall of the top of the column of erythrocytes in the given interval of time determines the erythrocytes sedimentation rate. The micro methods of estimation of ESR and PCV have been developed to overcome the drawbacks of macro methods, especially of taking blood sample by venepuncture. **Methods:** The present study was undertaken to compare ESR and PCV values as estimated by macro and micro methods in diseased and healthy subjects to define the cut off levels of ESR and PCV (Micro method) for the children and adults. When the mean values and the various percentiles obtained by micro method were compared with that of macro method, in healthy and diseased children, healthy and diseased male and female adults, none of them showed any statiscal significant difference. **Results:** The results of hematocrits by both methods on individual basis were compared. **Conclusion:** It was noticed that there was a close approximation of the readings in each case in micro and macro methods.

Keywords: ESR, PCV, Macro and Micro methods.

INTRODUCTION

The erythrocyte sedimentation rate is the rate at which erythrocytes settle down when anticoagulated blood is allowed to stand in a vertical column for a particular period (usually during 1st. hour) and the length of fall of the top of the column of erythrocytes in the given interval of time determines the erythrocytes sedimentation rate. ESR has more prognotic value than diagnostic value that is why this is being done commonly to monitor the course of the disease during the treatment: It is used to assist in the diagnosis of infections, inflammatory or neoplastic disease or serve as a guide in following the course of a disease.[2] An accelerated ESR is favoured by elevated level of fibrinogen and, to lesser extent alpha 2 beta, and gamma globulins. These asymmetric protein molecules have a greater effect than other proteins in decreasing the negativecharge of erythrocytes (Zeta potential) that tend to keep them apart. Thedecreased Zeta potential promotes the formation of rouleaux, which sediment more rapidly than individual, cells. Removal of fibrinogen by defibrination lowers the ESR.[18]

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Anaemia increases the ESR, because the change in theplasma ratio favoursrouleaux formation independent of changes in the concentration of the plasma proteiny any method of measurement, the ESR is most sensitive to altered plasma protein in the hematocrit range of 0.30 to 0.40.[5,15]

The quality of information obtained from the ESR is determined by the operating characteristics of the test and its sensitivity, specificity and predictive value. The determination of PCV was first introduced by Hadin in 1890. Later various modified methods were described but the technique described by Wintrobe using venous blood has gained almost universal acceptance and is regarded as one of the simplest and most reliable method.[10,16,20]

The micro methods of estimation of ESR and PCV have been developed to overcome the drawbacks of macro methods, especially of taking blood sample by venepuncture, which in certain categories of patients is difficult especially in children and obese subjects.[12]

Landau was the first to give detailed account of micro ESR,[12] performed in specially designed capillary tube and micro sedimentation rack. Later, other workers devised various micro methods for estimation of ESR and PCV.[7,11,13,22] In order that the results gain clinical significance it is mandatory that what ever method is chosen in the laboratory, it should be well standarized and its results, specially

the normal values in adults and children be known. Also the interpretation of higher micro ESR values must be determined by formulating a basic comparison amongst the three methods i.e. Micro, Wintrobe and Westergren methods.[11] The present study was undertaken to compare ESR and PCV values as estimated by macro and micro methods in diseased and healthy subjects to define the cut off levels of ESR and PCV (Micro method) for the children and adults.

Aims & Objectives

- To compare the values of ESR by three techniques i.e. Westergren, Wintrobe and Micro methods in health and disease in children and adults so that Micro method which requires only a drop of blood can be used as the method of choice in special cases, particularly in children.
- To determine the normal range of ESR for normal healthy children and adults by utilizing three techniques i.e.Westergren, Wintrobe and Micro Methods.
- To determine and compare the value of PCV by micro and macro haematocrit method in healthy and diseased children and adults.

MATERIALS AND METHODS

Study area.

In present study 300 cases were taken from Govt. Medical College Patiala alongwith school children from near by schools (healthy and diseased).

RESULTS

These cases were divided into four major groups i.e.

Group A - 50 healthy children
Group B - 100 healthy adults
Group C - 50 diseased children
Group D includes - 100 disease adults

Table 1(A): Age and Sex Distribution Group a (50 Healthy Children)

Age group (in years)	Male n=28	% age	Female n=22	% age
1-5	8	16	7	14
6-10	10	20	10	20
11-15	10	20	5	10

Table 1(B): Age and Sex Distribution of Group B (100 Healthy Adults)

Group (in years)	Male n=53	Female n=47
16-20	10	5
21-25	6	6
26-30	7	6
31-35	5	7
36-40	7	3
41-45	5	6
46-50	2	5
51-55	3	2
56-60	4	2
61+	4	5

Table 1(C): And Sex Distributio0f Group C (50 Diseased Children)

Age group (in years)	Male n=28	% age	Female n=22	% age
1-5	10	20	7	14
6-10	10	20	8	16
11-15	8	16	7	14

Table 1(D): Age and Sex Distribution of Group D (100 Diseased Adults)

Age group (in years)	Male n=52	Female n=48
16-20	11	5
21-25	4	5
26-30	8	7
31-35	7	5
36-40	4	6
41-45	6	6
46-50	4	5
51-55	1	-
56-60	2	2
61+	5	7

Table 2: Distribution of the Morbid Conditions among Diseased Children

Sr. No	Morbid Condition	Number	% age
1	Tuberculosis	8	16
2	Chronic Respiratory infections	10	20
3	Gastro-enteritis	8	16
4	P.U.O	6	12
5	Lymphademtis	5	10
6	Seizure	3	6
7	Others	10	20
	Total	50	

Table 3: Distribution of Morbid Conditionsamong Diseased Adults

Sr. No	Morbid Condition	Number	% age
1	Chronic inflammatory disease	18	18
2	Tuberculosis	26	26
3	Chronic infective disease	20	20
4	P.U.O	9	9
5	Lymphadenitis	7	7
6	Pelvic inflammatory disease	9	9
7.	Non haematological malignancy	3	3
8.	Others	8	9
	Total	100	

Table 4: Mean Value of ESR by the Micro Westergren and Wintrobe Methods in Differentage Groups in Healthy Subjects

Age in years	No. of cases	ESR Estimation by		
		Micro	Westergren	Wintrobe

		Mean	±SD	Mean	±SD	Mean	±SD
1-5	15	7.73	2.01	8.06	1.76	7.46	1.78
6-10	20	6.9	2.14	7.2	1.91	6.4	1.85
11-15	15	8.00	2.60	8.6	3.28	7.66	2.64
16-20	15	6.8	1.75	6.8	1.55	7.0	1.59
21-25	12	6.9	1.11	7.66	1.24	6.66	0.94
26-30	13	7.84	1.02	8.30	1.26	7.23	1.12
31-35	12	7.0	2.08	7.25	2.55	7.16	2.15
36-40	10	10.5	1.02	11.4	1.01	12.1	1.04
41-45	11	8.09	0.79	8.9	0.79	7.18	0.71
46-50	7	9.14	1.18	12.85	2.09	11.42	2.06
51-55	5	11.2	0.72	12.4	1.01	9.6	0.8
56-60	6	9.16	1.06	10.5	0.95	8.33	0.94
61-65	4	10.25	1.29	13.0	1.58	9.5	1.11
>66	5	11.2	0.74	12.2	0.4	12.6	0.8
Total	150						

Table 5: Shows the Cut off Values of ESR Estimation by Different Methods in Normal Population

Sr. No	Subjects	Westergren (A)	Wintrobe (B)	Micro (c)
1.	**Children**			
	Mean + 2SD	12.76	11.46	12.1
	90th Percentile	10	10	10
	95th Percentile	14	11	13
2.	**Adult Males**			
	Mean + 2 SD	14.67	13.76	12.72
	90th Percentile	13	12	12
	95th Percentile	13	14	13
3.	**Adult Females**			
	Mean + 2SD	14.4	13.51	12.67
	90th percentile	14	13	12
	195di percentile	14	14	13

Table 6: Shows the Reliability of Micro Method of Esrestimation Taking 95th Percentile Value as Cut Offlevels against Westergren and Wintrobe Methods Asstandard

Sr. No	Subjects	Reliability of Micro method of ESR		
		Sensitivity	Specificity	Positive Predictive value
1.	**Children**			
(a)	Westergren	94%	94%	94%
(b)	Wintrobe	90%	98%	97.8%
2.	**Adult Females**			
(a)	Westergren	70%	100%	100%
(b)	Wintrobe	100%	92%	92.59%
3.	**Adult Males**			
(a)	Westergren	100%	92%	92.59%
(b)	Wintrobe	100%	90%	90.09%

Table 7: Compares the Average Values of PCV in Healthychildren by Macro and Micro Methods (N - 50)

PCV (%)	Methods	
	Macro	Micro
Mean	46.64	47.02
S.D.	3.94	3.86
Range	36-52	35-52
Median	48	49

Table 8: Compares the Average Values of PCV in Healthyadults Male by Macro & Micro Method (n = 53)

PCV (%)	Methods	
	Macro (Wintrobe)	Micro
Mean	43.32	43.66
S.D.	4.36	4.61
Range	36-51	35-50
Median	45	46

Table 9: Compares the Average Values of PCV in Healthy Adultfemales by Macro and Micro Methods (N = 47)

PCV (C/c)	Methods	
	Macro (Wintrobe)	Micro
Mean	45.59	45.53
S.D.	4.11	4.26
Range	36-53	35-53
Median	46	47

Table 10: Compares the Average Values of PCV in Disease Children by Macro and Micro Methods (n=50)

PCV (%)	Methods	
	Macro (Wintrobe)	Micro
Mean	40.24	39.46
S.D.	4.88	4.77
Range	31-49	31-48
Median	41	41

Table 11: Compares the Average Values 01p, V in Diseasedadults Male by Macro and Micro Methods (n = 52)

PCV (%)	Methods	
	Macro (Wintrobe)	Micro
Mean	40.86	40.63
S.D.	5.80	5.97
Range	31-52	30-52
Median	42	41

Table 12: Compares the Average Values of PCV in Diseased Adultfemales by Macro and Micro Method (n = 48)

PCV (%)	Methods	
	Macro (Wintrobe)	Micro
Mean	39.95	39.35
S.D.	7.35	7.32
Range	25-56	23-55

Median	40	40

Table 13: Compares the Mean Difference in PCV on Children Asestimated by Macro and Micro Methods

PCV (%)	Methods	
	Macro & Micro (Healthy) n=50	Macro & Micro(Diseased) n=50
D	0.38	0.78
S	1.52	2.84
Student t' (paired testvalue)	1.80	1.95
P > 0.05 (Insignificant)		P> 0.05 (Insignificant)

Table 14: Compares the Mean Difference in PCV on Male Subjectsby Macro and Micro Methods

PCV (%)	Methods	
	Macro & Micro (Healthy) n= 53	Macro & Micro(Diseased) n = 52
d	0.34	0.23
S	1.31	0.91
Student 't' (paired test value)	1.88	1.91
P>0.05 (Insignificant)		P>0.05 (Insignificant)

Table 15: Compares the Mean Difference of PCV in Adult Femalesas Estimated by Macro and Micro Methods

PCV(%)	Methods	
	Macro & Micro (Healthy) n=47	Macro & Micro Diseased)n=48
d	0.06	0.6
S	1.03	2.16
Student 't' (paired testvalue)	0.39	1.93
P> 0.05 Insignificant		P > 0.05 Insignificant

DISCUSSION

Comparison of ESR by Westergren, Wintrobeand Micro Methods in Normal Healthy Population

In children

In present study, the mean value of ESR in this group of 50 healthy children was 7.46 ± 2.32 mm by micro method. This value was between the higher mean (7.88±2.44mm) in Westergren method and lowest (7.1±2.18 mm) in Wintrobe method (table-IVa). The higher range of ESR in this group was noticed in Westergrennethod (13.00mm) followed by Wintrobe (12.6 mm) and micro method (11.2mm) (The mean difference in ESR value between Westergren and Micro method was 0.4Z mm. 1st hour i.e micro method gave slightly lower reading for ESR in healthy children and the differee was statistically significant $p < 0.05$ when tested by student paired 't' test

In adults

The mean value of ESR in healthy men by Wintrobe method was (8.16±2.80 mm, 1st hour) which was comparable to mean value by Micro method (8.30±2.81 mm), but the mean ESR by Westergren method (9.11±2.78mm 1st hour) was slightly higher than micro and Wintrobe method of ESR estimation.
The mean ESR value in healthy female was highest in Westergren method (9.42±2.49 mm 1st hour) while the value in other methods were comparable (8.53±2.30 and 8.61±2.03 mm 1st hour).
The median value of ESR in healthy males was 9 mm in Westergren metho7 and 8 mm in Wintrobe and Micro methods respectively. In healthy females, the ESR was same as in male by Westergren method (9 mm 1st hour) and both by Wintrobe and Micro methods it was the same (8 mm 1st hour). By comparing in healthy adults, the ESR values in Westergren method were slightly higher than the values by Wintrobe and Micro methods and these were comparable. All these differences were statistically insignificant.

Comparison of ESR by Westergren, Wintrobe OBE Andmicro Methods in Diseased Population

Children

Among the diseased children 5th, 10th percentile values for ESR asestimated by the three methods were comparable. There after, the Westergren method of ESR estimation gave highest values and micro method gave the lowest values.
The mean value of ESR by Westergren was 32.6±17.5 mm 1st hour, by Wintrobe 27.7±12.7 mm 1st hour and by micro method 22.62±9.67 mm 1st hour.
The median values were variable by only 1 mm in Westergren and Wintrobe. The lower limit in the range of ESR was same (8 mm). While the upper limits, showed marked variations. (85,54,42 mm) in Westergren, Wintrobe and micro methods respectively. The mean difference in ESR value between Westergren and Micro method in diseased children was 9.78 mm 1st hour and this was statistically significant. The mean difference in the ESR values in diseased children between Wintrobe and Micro and between Westergren and Wintrobe was 5.12mm 1st hour and 4.9 mm 1st hour, respectively and were also statistically significant.

Adults

After evaluating the results of ESR by the three methods in diseased adults, both males and females, it was observed that in general the ESR reading was highest in Westergren followed by Wintrobe and Micro methods.
The range of ESR in the three methods was variable. It was maximum in Westergren. The lower limit of the reading in male and female was 15 mm 1st hour.
The upper limit of ESR in diseased subjects showed marked Variation i.e. in Wstergren it was 122mm in males and 115mm in females. Whereas inWintrobe,

it was 57 and 60 mm and in micro, it was 45 mm and 42 mm in males and females respectively.

In an attempt to evaluate the practical significance of Micro ESR, the control and study group patients were classified in arbitrary groups of progressively rising ESR as shown in table XX. It was observed that subjects of the control group showed the ESR 15 mm 1st hour or below (i.e. below the normal cut off value of Micro ESR). Only three were in the range of 16-20mm Ist hour.

Range of ESR in Con3ol and Study Group

m-ESR	Control group	Study group
0-5	35	-
6-10	73	5 (children)
11-15	39	5 (Children)
16-20	3	41
21-25	-	44
26-30	-	26
31-35	-	15
36-40	-	11
41-45		3
Total	150	150

It has been observed that Westergren method showed more difference than the Wintrobe method while comparing with the Micro ESR method. It was observed that when Micro ESR values were in the range of 0 to 10 mm the difference did not exceed 2 mm in any case. The value of Micro and Macro methods are well correlated at lower readings and not when they are higher.

The observations in the present study are similar to those observed by various workers. There is by and large a good correlation between the results of the three methods in the normal range of the ESR and when the values are low, but when the ESR is raised as in diseased cases, the correlation amongst the readings in the three methods' in the same patient decreased.

In 1933 Landau carried out ESR measurement in 300 cases (including children, adults) by micro method and Westergren method. He concluded that between the low normal range, of 1 to 8 mm of ESR, the difference in the two methods did not exceed 2 mm. While the results were much different when the ESR values were high. In order to correlate the readings in the 2 months, Landau grouped them arbitrarily and found out that the values in micro ESR of 8,15,25,30 and 45 mm 1st hour corresponded with 8,18,40,70 and 150 mm 1st hour in Westergren method.

Packed Cell Volume (PCV)

The micro method for PCV estimation is well established as a standard method. The difference in results is due to variable amount of trapped plasma volume in different methods. In the standard macro method using Wintrobe tube, the hematocrit are expected to be a little higher due to more plasma trapping.

Some studied are available in which results of micro and macro methods of PCV estimation have been compared.

Mclnroy studied 50 cases and found no significant difference between the micro and Wintrobehematocrit.[14]

McGovern et al,[13] compared the micro and Wintrobe hematocrit on 100 infants and children. They concluded that micro hematocrit levels closely approximated with the Wintrobe hematocrit levels. The correlation coefficient between the values obtained by micro and Wintrobe hematocrit was very high (0.933). The Wintrobe method yields a significantly higher packed cell volume than micro hematocrit method ($p < 0.001$). Though the difference between the means was statistically significant, it was so small, that it beared no practial significance. They concluded that PCV in the range of 35 to 55 % as determined by micro hematocrit, did not differ from predicted Wintrobe PCV by more than 2 unit. So that the micro hematocrit level of capillary blood is 35 % thepredicted Wintrobe value was 37.0 %, the micro hematocrit value of 55%, was correlated with 54.6% of Wintrobe hematocrit.

Brecher et al,[3] and Brittinet al,[4] compared the manual (micro/macro method) and the electronic counter results and found that the hematocrit readings could be linearly correlated in both methods within the normal range.

Faribanks compared the results of Erythrocyte indices including the hematocrit by the manual (micro/macro methods) and the automated counter.[8] He changed the R.B.C counts by dilutingthe blood or using the centrifuged blood.It was noticed that at extreme ends,theM.C.Vvaried remarkably and soalso thehematocrit values. The wide rangeof RBC countprepared in this study was from 0.8 to 9.4 x 1012/ liter, the hematocrit values did not linearly correlate at the extreme values. But in the range of 25 to 50% (hematocrit clinical range) the results 'of all methods showed close approximation.

Young et al,[21] estimated the P.C.V. in 66 children by micro method and the Coulter electronic counter. On evaluation they found that the sensitivity of the micro hematocrit was 90% the specificity was 43.5. They concluded, micro hematocrit method using capillary blood, will miss very few patients with significantly lo venous hemoglobin values and is thus an acceptable screening test for anemia.

The present study was aimed to compare the micro haematocrit results with those observed by the macro method in the same persom So that apractically convenient method employing very little finger prick capillary blood can replace the macro methods in the routine laboratories. In the total 300 cases, which included 50 healthy children, 50 diseased children, 100 healthy adults and 100 diseased adults of both sexes, the double hematocrit tests revealed only minor differences in different study groups. This difference were statistically insignificant in all the

cases. This would be more true when the same method is applied for follow up of affected patients.

Comparison of the mean value of the micro and macro hematocrit of different study groups was done and it is found that the me n values of the results in the,2 methods are almost similar in each ca s fig (There is also not much difference in the diseased and healthy subjects When compared by sex and age group. This is So because in the present study the patients having hematological problems were excluded. Hence no variation in the hematocrits from the normal range was noticed in any case.

There are statistically significant variation in the values of ESR by micro method, Westergren & Wintrobe method, especially when the ESR is high. The Westergren which is the standard and most reliable method, is being used in most of the laboratories and institutions. This study also recommend the use of this method in future.

There is close approximation in the values of haematocrit in micro and macro methods. So any method can be used in laboratories and institutions, depending upon the facilities available.

CONCLUSION

The present study was carried out on 150 disease subjects and 150 healthy subjects. The blood samples were collected by venepuncture and capillary blood from pulp of finger as per the standard technique. The ESR estimation was done by Westergren and Wintrobe methods and micro techniques, and PCV by Wintrobe and micro methods.

In the healthy control group.the mean values of ESR, by the Westergren method was highest followed by the micro ESR and that observed in the Wintrobe method. The value of ESR by Westergren, micro &Wintrobemethod were 7.88±2.44, 7.46±2.32 and 7.10±2.18mm first hourrespectively. In the 53 healthy males, it was 9.11±2.78, 8.30±2.21 and 8.16±2.80 mm 1st hour and in the 47 healthy females it was 9.42±2.49, 8.61±1.03 and 8.53±2.30 mmfJ\st hour by the Westergren, micro and Wintrobe methods respectively.

On mutual comparison by the students paired 't' test of the results obtained by the 3 methods in the healthy children group, it was noticed that though the differences were of minor degree. In the male and female healthy adult groups, a similar comparison showed no statistically significant difference when the results of micro and Wintrobe methods were compared. But when the results of Westergren were compared with the Other two methods, the difference was statistically significant.

When the reliability of micro ESR was evaluated against the standard Westergren method, with the cut off value of mean +2SD, it was noticed that the micro ESR had a sensitivity of 98%, 100% and 100% and specificity of 96%, 92% and 96% in children, adult males and females respectively. The predictive value varied form 92.59% '100% in the 3groupsof patients. All the measures of reliability were also high whenmicromethod was comparedwith Wintrobe method. The reliability ofmicro ESR was also high when the cut off values of 90th and 95th percentile were used.

In the present study when the mean values and the various percentilesobtained by micro method were compared with that of macro method, in healthy and diseased children, healthy and diseased male and female adults, none of them showed any statiscal significant difference.

The results of hematocrits by both methods on individual basis werecompared. It was noticed that there was a close approximation of the readings in each case in micro and macro methods.

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