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A COMPARISON OF SOME SIMPLE TECHNIQUES OF HAEMOGLOBIN MEASUREMENT

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SUMMARY. In view of the reported high prevalence of anaemia among pregnant women, a valid, affordable and easy to use screening procedure is essential for its detection. The relative usefulness of the Talliquist and Sahli's methods of haemoglobin estimation have been compared with the cyanmethaemoglobin method. The Talliquist method was found to have low reliability. The accuracy was higher in Sahli's method in comparison to Talliquist method. When a cut off of 10 or 11 gms Hb/dl was used, the sensitivity of both Talliquist and Sahli's methods were identical, although the specificity behaved differently. In this respect Sahli's method does not seem to have any advantage over the Talliquist method as a screening procedure in the detection of anaemia in the population group studied. However, in the present study only 5.8% of the population had a haemoglobin value below 9 gms% (as recorded by the cyanmethaemoglobin method). It is therefore necessary, to investigate further the validity of these techniques in a more anaemic population.

Key Words: Haemoglohin estimation, Talliquist, Sahli and cyanmethaemoglobin methods

INTRODUCTION

"Almost half the women in the developing world are anaemic - and among pregnant women that figure rises closer to 60%"(1). As a practical approach to the characterisation of anaemia a WHO scientific working group has suggested an arbitary cut off point of 11g haemoglobin (Hb) per decilitre, for adult pregnant women living at sea level (2).

"In Sri-Lanka the main source of information on the prevalence of anaemia amongst women are the Maternal and Child Health (MCH) clinics. The reported prevalence rates among pregnant and lactating women range from 65-80%" (3). It is therefore extremely important that anaemic women be detected early in pregnancy and appropriate therapy be instituted. To achieve this a suitable method must be made available to the field worker. Such a method should be valid, affordable and easy to use. The method presently in use at MCH clinics is the Talliquist card.

Although the repeatability of different techniques used in the estimation of blood haemoglobin levels have been studied under laboratory conditions (4, 5), in the assessment of a screening procedure, the validity of the technique has to be studied in a given epidemiological situation.

This study was undertaken to evaluate the relative usefulness of the Talliquist method, in comparison to Sahli's method, both of which involve visual colour matching. Sahli's method was included in the study as it was thought to be an affordable alternative to the currently used Talliquist's method, at field level. The value obtained using the cyanmethaemoglobin method was used as the standard.

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METHODS

The study was carried out in the antenatal clinics of the Medical Officer of Health area, Kotte. The University has been providing the services of a laboratory teehnician for the antenatal clinics held at the Pitakotte Health Centre and this technician carries out the routine Hb estimations of all mothers at the first visit, using Sahli's method. All such mothers needing haemoglobin estimations on a given day were included in the tudy. Four consecutive clinic days yielded a sample of fifty two mothers. All three methods studied used capillary blood and blood from the same finger prick was used for all tests.

Two observers, that is, two midwives who remained observer I and observer II throughout the study, carried out two independant estimates using the Talliquist method. Two laboratory technicians carried out the Sahli's test and the cyanmethaemoglobin technique. Each test was done by the same observer on all occasions.

In the Talliquist method, a drop of whole blood is placed on a strip of blotting paper and, when the sheen of the blood has disappeared, the colour is compared visually with a colour standard on paper. The Hb concentration is read off from the values corresponding to the closest matched colour standard.

In Sahli's method, the graduated tube provided is filled up to the 20 mark with a solution of 0.IN HCl A sample of 0.02 ml. of whole blood taken with a standardized manual pipette is added to this solution and the pipette rinsed in the solution. This is allowed to stand exactly for five minutes. The standard provided with the kit is placed by the side of the dilution tube, and distilled water is added with a dropper until the acid solution matches the standard. The Hb concentration is read off from the graduations on the tube and is given as a percentage. Here 14 gm Hb/dl is taken as 100%.

In the cyanmethhaemoglobin method 0.02 ml of blood is taken using a standardised manual pipette and is added to 5 ml of Drabkin's reagent. The tube containing the solution is stoppered and inverted several times. After allowing it to stand at room temperature for 3-10 minutes to ensure complete reaction, it is compared with the haemoglobin standard in a Klett-Summerson photoelectric colorimeter with a suitable filter (540nm) against a reagent blank. The cyanmethaemoglobin standard, which is supplied in a sealed ampoule and where the haemoglobin concentration is given, is read in a similar manner against the reagent blank.

$$\text{Blood Hb gm/dl} = \frac{\text{optical density of sample}}{\text{optical density of standard}} \times \frac{\text{dilution factor} \times \text{conc. of standard}}{1000}$$

The dilution factor is dependant on the volume of reagent used. In this experiment, as 5ml of reagent was used ,the dilution factor was taken as 251.

RESULTS

The frequency distribution of the haemoglobin estimates made by the two observers using the Talliquist method is shown in Table 1. The results indicate that observer II appears to read lower values than observer I and also within a narrower range (as indicated by the lower SD).

TABLE 1. Frequency distribution of Hb concentration by Talliquist method

Hb conc. g/dl	Observer I	Observer II
8—	5 (9.6%)	10 (19.2%)
9—	29 (55.8%)	34 (65.4%)
10—	9 (17.3%)	5 (9.6%)
11—	7 (13.5%)	3 (5.8%)
12—13	2 (3.85%)	0
Total	52 (100%)	52 (100%)
Mean	9.46	9.01
SD	1.08	0.85

This difference is also demonstrated in Table 2, where the mean haemoglobin concentration of each observer for a given value of the standard (obtained by the cyanmethhaemoglobin method) is examined. The mean values recorded by observer I are higher than those obtained by observer II except at the two extremes, where the numbers of patients included were small.

TABLE 2. Mean haemoglobin concentrations recorded by the two observers using Talliquist method for given value of the standard.

Hb conc. of standard		Observer I		Observer II	
g/dl	n	mean	sd	mean	sd
8—	3	8.58	0.78	9.10	0.46
9—	14	9.24	0.96	8.63	1.08
10—	12	9.68	1.07	9.29	0.78
11—	10	9.85	1.46	9.04	0.84
12	8	9.36	0.59	9.06	0.83
13—	4	9.55	1.33	9.16	0.39
14—15	1	8.58	—	9.36	—

The repeatability of the Talliquist method was examined using the correlation between replicate measurements made by the two observers on the same subject. The correlation coefficent was 0.3754 ($p<0.0061$), indicating that the method has very low repeatability; that is, the random measurement error is high when compared to the true variation seen in the population.

The repeatability of a measurement is improved by taking the mean of two or more readings. Hence the mean values of the two Talliquist readings were used in the measurement of its validity in the detection of 'anaemia'.

Comparison of the frequency distributions of the Hb estimates made by the Talliquist method, Sahli's method and the cyanmethaemoglobin method are given in Table 3. The results show that Sahli's method gives lower values of Hb estimates. The mean value is lowest for Shali's and highest for the cyanmethaemoglobin method In the Talliquist method all the readings appear to be concentrated within a very narrow range of 8.58-9.85. Analysis of variance showed that the differences observed between the groups are highly significant (Table 4).

TABLE 3. Frequency distribution of haemoglobin concentration by the three methods used in the study

Hb conc g/dl	Talliquist	Sahli's	Cyn. Meth. Hb.
6—	—	3 (5.8%)	
7—	—	5 (9.6%)	
8—	9 (17.3%)	17 (32.7%)	3 (5.8%)
9—	26 (50.0%)	19 (36.5%)	14 (26.9%)
10—	13 (25.0%)	8 (15.4%)	12 (23.1%)
11—	4 (7.7%)		10 (19.2%)
12—			8 (15.4%)
13—			4 (7.7%)
14—15			1 (1.9%)
Total	52 (100%)	52 (100%)	52 (100%)
Mean	9.24	8.46	10.44
SD	0.80	0.97	1.46

TABLE 4. Anova table

	df	ss	ms	F
between groups	2	103.644	51.8222	41.79
within groups	153	189.752	1.2402	
Total	155	293.396	1.8929	

($F_{2,153} = 4.61$)
Therefore F is highly significant.

Table 5 compares the mean values of haemoglobin concentrations obtained with the Talliquist method and Sahli's method, corresponding to given values of the standard, which is the cyanmethaemoglobin method. It is seen that both Talliquist and Sahli's methods read lower than the standard, except in the range 8-8.99 gm/dl, where the Talliquist method appears to read higher. This should however be interpreted with caution, as the numbers in this group are very small.

TABLE 5. Comparison of the mean haemoglobin concentrations obtained by Talliquist and Sahli's methods corresponding to given values of the standard.

Hb conc. of standard		Talliquist		Sahli's	
g/dl	n	mean	sd	mean	sd
8—	3	8.84	0.60	6.53	0.40
9—	14	8.94	0.90	7.81	0.76
10—	12	9.49	0.77	8.42	0.66
11—	10	9.45	0.97	8.79	0.68
12—	8	9.21	0.46	9.20	0.33
13—	4	9.36	0.84	9.83	0.29
14—15	1	8.97	—	8.82	—

TABLE 6. Comparison of the mean differences in haemoglobin concentration obtained by Talliquist and Sahli's methods, corresponding to given values of the standard

Hb conc. of standard		Talliquist		Sahli's	
	n	mean	sd	mean	sd
g/dl		diff		diff	
8—	3	—0.92	0.82	1.38	0.51
9—	14	0.10	0.78	1.23	0.77
10—	12	0.58	0.75	1.64	0.57
11—	10	1.59	1.01	2.25	0.57
12—	8	2.78	0.59	2.79	0.47
13—	4	3.56	0.77	3.08	0.55
14—15	1	4.55	—	4.70	—

Table 6 shows that the mean difference in Hb concentration obtained by the two methods for given values of the standard, tends to increase with increasing haemoglobin concentration, i.e. the discrepancy between the standard and the method increases with increasing haemoglobin concentration.

The accuracy of a technique depends on the difference between each individual measurement and the relevant reference value. The accuracy of each of the methods of haemoglobin estimation studied is indicated in Figs. 1 and 2. These show the scatter of the individual measurements obtained with each method, in comparison to those of the standard, the corresponding regression lines and their 95% confidence intervals. The 95% confidence intervals were calculated from the standard error of the estimates and its width indicates the variability of accuracy. This is seen to be much wider in the Talliquist method than in Sahli's method.

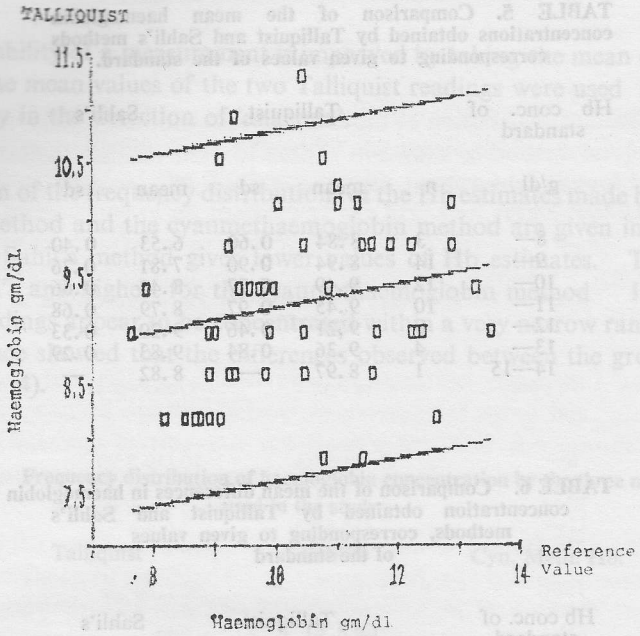

Fig. 1 Scatter diagram of all measurements made with Talliquist (mean of two readings), regression line and 95% confidence belt.

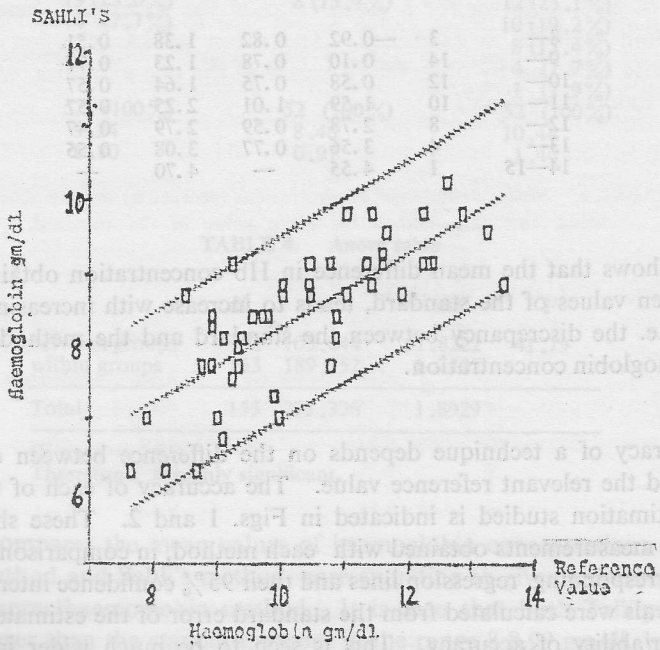

Fig. 2 Scatter diagram of all measurements made with Sahli's method, regression line and 95% confidence belt.

The adequacy of the regression model was tested by analysis of the residuals. This showed no curvilinearity or a change in residual variance with increase in the predicted value of y. This indicates that the assumption that "the relationship between each technique and the standard is linear" holds true, that is, a regression line is an appropriate method of expressing the relationship between these variables.

The validity, that is, the sensitivity and specificity of each of the methods as a screening procedure in the detection of anaemia, would depend on its ability to detect the concentration of Hb in a sample of blood as being above or below a given value, which is the 'cut off' point for making the decision whether a person is anaemic or not.

The sensitivity and specificity of the Talliquist and Sahli's methods when different levels of Hb are taken as "cut off" points, are indicated in Table 7. This shows that there is no difference at all in the sensitivity between Talliquist and Sahli's methods at the three "cut off" points tested. On taking a value of 9 gms haemoglobin/100 ml as the "cut off" point, the ability of each of the tests to detect anaemia is seen to be considerably low.

TABLE 7. Sensitivity and specificity of Talliquist and Sahli's methods, when different values of the standard are used as the cut off point.

Cut off Hb conc.		Talliquist	Sahli's
9 gms/dl	sensitivity	33.30%	33.30%
	specificity	44.90%	85.71%
10 gms/dl	sensitivity	82.35%	82.35%
	specificity	40.00%	22.86%
11 gms/dl	sensitivity	89.66%	89.66%
	specificity	4.35%	0.00%

In general, the specificity of each of the tests as indicated by its ability to distinguish those who are not anaemic as in fact being so, is low. The exception to this observation was when Sahli's method was used to identify those who are not anaemic taking the lower 'cut off' of 9 grams/100 ml.

In the study group there are only a few women with haemoglobin levels below 9 grams/ 100 ml. Therefore the characteristics of the tests described above using this 'cut off' have to be interpreted with caution.

DISCUSSION

Several factors are known to influence the repeatability of a measurement. They include biological variation, factors related to the instrument and those related to the observer. The very low correlation coefficent (0.3754) that was observed between the two readings obtained by observer I and II using Talliquist method, indicates that the method has very low repeatability ; that is, the random measurement error is high when compared

to the true variation seen in the population. This points to the need to standardise the technique as far as possible, as well as the need for cautious interpretation of the actual values obtained.

The accurracy of the two methods are shown in Figs 1 and 2 and, as expected with low reliability, the accuracy of the Talliquist method is much lower (Fig 1) than that of Sahli's method (Fig. 2).

The validity of a method would depend on both specificity and sensitivity. Using these criteria the validity of both tests studied is rather low. However, in the field situation in which the tests are used, the important aspect would be the ability of the test to detect as anaemic, most of the women whose haemoglobin levels are below a pre-determined 'cut-off' point, i.e. a high sensitivity. If in this process some women who are in fact not anaemic, are identified as anaemic, as would occur with a test with low specificity, it is unlikely to provide a negative impact on the services directed towards management of anaemia in pregnancy. In this respect Sahli's method does not seem to have an advantage over the Talliquist method.

However, before any conclusions are drawn it is important to study further the behaviour of the Talliquist method in an anaemic population, especially because of the indication that at lower levels of haemoglobin concentrations it may read higher than the standard or true value. Therefore the validation procedure should be carried out in a population representative of all ante-natal mothers.

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