Variability And Accuracy Of Sahli's Method In Estimation Of Haemoglobin Concentration

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Abstract: Background: Haemoglobin concentration provides information about the status of anaemia in the population. Haemoglobin estimation is the routine and frequently performed screening haematological test of laboratory services. The Type of methods used and sites of collection of blood samples has found to make differences in haemoglobin concentration. The methods used in routine measurement are needed to be adjusted to obtain comparability with the haemiglobincyanide method. So the present study was undertaken with a objective to test the reliability of Sahli's method in estimation of haemoglobin concentration and standardizing it in district hospital laboratory of Dhule in Maharashtra. Material & Methodology: Haemoglobin concentration (gm/dl) in blood is compared in 173 apparently healthy medical college students within 18 to 23 years age. Results from Sahli's method were compared to Haemiglobincyanide (HiCN) method within capillary and venous blood of same subjects. Results: Sahli's method has lower values than Haemiglobincyanide method with mean difference of 0.62gms/dl(95%CI; 0.51 to 0.73, p<0.01) in capillary blood and 1.1gms/dl(95%CI; 0.92 to 1.26, p<0.01) in venous blood. By Sahli's method's ability to diagnose anaemia has sensitivity of 83.7% & 90% and specificity of 63.2% & 60.2% in capillary & venous blood respectively. Sahli's method has significant (p<0.01) positive correlation coefficient in capillary blood & venous blood. Variability of haemoglobin concentration in Sahli's method is less in capillary blood then venous blood in caparison to HiCN method. Interpretation & conclusion: Sahli's method had lower values of haemoglobin in capillary and venous blood compared to HiCN method. Haemoglobin concentration was lower in capillary blood than venous blood by both methods. For Accuracy of Sahli's method the correction factor should be considered to ensure comparability of results with the reference method.[Patil P et al NJIRM 2013; 4(1) : 38-44]

Key Words: Haemoglobin, Sahli's Method, Haemiglobincyanide Method, Dhule.

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Introduction: Haemoglobin is the iron-containing protein in red blood cells. Reduced haemoglobin below normal leads to 'Anaemia'. It is caused by nutritional deficiencies commonly, also by acute and chronic inflammation, parasitic infestations and inherited or acquired disorders. Haemoglobin concentration provides information about the status of anaemia in the population.

Haemoglobin estimation is the routine and frequently performed screening haematological test of laboratory services. The world health organization has define the lower limit of normal for haemoglobin concentration at sea level to be 12.0 gm / dl in women and 13.0 gms/dl in men[1]. In general practice measuring haemoglobin is a valuable primary and sensitive screening test that gives reliable prediction on the need to do full blood count.[2]

Haemoglobin content of blood in solutions may be estimated by several methods, by measurement of its colour, erythrocyte volume fraction, specific gravity or its iron content. The methods are colour or light intensity matching techniques that measure inert pigments in blood with different degree of efficiency.[3]

The type of method is selected based on its feasibility, cost effectiveness, simplicity, reliability, and easy to use in the laboratory and in the field [4]. Type of method and site of blood sample [5] used is found to make a significant within subject variability of haemoglobin concentration.[4, 6, 7, 8, 9]

Sahli's method of haemoglobin estimation is relatively inexpensive, simple to use, does not require electricity & requires only small sample of blood. In developing country like India most common method used for haemoglobin estimation is the Sahli's method. Photometric determination of Haemiglobincyanide (HiCN) is recommended as the reference method for haemoglobinometry in human blood by International Committee for

Standardization in Hematology (ICSH standard 1995). All other methods used in routine measurement are needed to be adjusted to obtain comparability with the haemiglobincyanide method.[10]

Keeping in account high cost, and lack of facilities in the developing countries the use of sophisticated equipments is limited for screening of haemoglobin. Because of observer's error, instrumental errors and cost effectiveness of Sahli's method we need to bridge the results variability and accuracy. Also it is not sufficiently accurate because it shows wide scatter in inter observers and intra observer results distribution.[6, 8]

The reliability of the method needs to be confirmed as unreliability widens the distribution of haemoglobin values and results in biases in estimates of prevalence of anaemia and response to intervention for it. The present study was carried out to determine the accuracy and variability of results by Sahli's method compared to Haemiglobincyanide method in estimation of haemoglobin in screening and laboratory diagnosis. Hence the present study was undertaken for standardizing the haemoglobin results in district hospital laboratory.

Material and Methods: Present study was conducted by department of Physiology in Shri Bhausaheb Hire Government Hospital and Medical College, Dhule in tribal belt of Maharashtra. From Apparently healthy medical college students within 18 to 23 years of age 173 of those voluntary participating in the study were randomly selected. Their haemoglobin estimation was done in the District Civil Hospital Laboratory at Shri Bhausaheb Hire Government Medical College. The blood for the test was collected from a finger prick and intravenously, after obtaining written informed consents of the subjects. Study was permitted by institutional ethical committee.

Screening laboratory in District hospital performs haemoglobin estimation of outpatient department by Sahli's Method where the Capillary blood is collected by finger prick method. For indoor patients the venous blood is collected by venepuncture and haemoglobin estimation is done by Sahli's method in central laboratory.

Collection of Blood: Two trained laboratory technicians were instructed thoroughly according to the standard operating procedure provided to them. The subject was called at screening laboratory and with all aseptic precautions 20 µl of capillary blood was collected by finger prick from ring finger of left hand for Sahli's method, following this 20 µl blood was collected and put in labeled test tube filled with 5ml Drabkin's solution for Haemiglobincyanide method.

From the same subject again 2 ml venous blood was collected from left cubital vein in test tube containing ethylenediamine tetra acetic acid (EDTA). Venous blood was sent to the central laboratory for estimation of haemoglobin by Sahli's method and Haemiglobincyanide method.

Sahli's Method[11]:-

The haemoglobin tube (STD 14.5gm = 100% concentration) was filled with N/10 hydrochloric acid (HCL) upto 2 gm marking. This graduated tube was placed in Sahli's Hemoglobinometer (Comparator with Brown glass). Blood sample obtained from capillary or venous blood was drawn in Sahli's pipette up to 20µl mark and added in haemoglobin tube containing N/10 HCL. The blood and acid are mixed with glass stirrer and allowed to stand for 5 minutes for acid haematin formation. Drop by drop distilled water was added to dilute the acid haematin compound colour till it matches with the standard colour plates of the comparator. Results were read as gms/dl present on the haemoglobin tube.

Haemiglobincyanide method[11]-Blood was diluted in the ICSH reagent based on Drabkin's diluting fluid that contains potassium ferricyanide, potassium cyanide and a non-ionic detergent. Red cells are lysed and the released haemoglobin was converted to Haemiglobincyanide. 20 µl of blood was added to 5 ml Drabkin's diluting fluid collected by automated dispenser. Reading was taken on

calorimeter with green filter. Haemoglobin values were calculated by using the formulae[11]

Hb (g/l) = (Test/Standard) x Conc. of standard (mg/l)* x (251/100)
* Concentration of Standard = 60 mg/100ml.

Table No 1: Gender wise distribution of subject within age(n=173)

Age (Years)	Gender.		Total
	Female	Male	
18	10 (34.5%)	19(65.5%)	29(16.8%)
19	9(40.9%)	13(59.1%)	22(12.7%)
20	19(37.3%)	32(62.7%)	51(29.5%)
21	14(66.7%)	7(33.3%)	21(12.1%)
22	14(35.0%)	26(65.0%)	40(23.1%)
23	5(50%)	5(50%)	10(5.8%)
Total	71(41.0%)	102(59%)	173(100%)

In order to reduce procedure bias, technician performing the procedures was blinded of the reason and purpose of investigation, to keep the procedure as routine as possible. Same investigator had estimated haemoglobin concentration by Sahli's method or haemiglobincyanide method on capillary and venous blood and the results were recorded individually.

Analysis- The results obtained were analyzed using Statistical package for social science software version 16 (SPSS 16). Paired t test was used to compare values obtained by Sahli's method with Haemiglobincyanide method which served as control for capillary blood and venous blood.

Accuracy of Sahli's method was assessed according to sensitivity, specificity , positive and negative predictive values(PPV & NPV), Percent False Positivity (PFP) & Percent false negativity(PFN) in diagnosing anaemia (Haemoglobin Cut off levels as per WHO guidelines[(1)]) compared to haemiglobincyanide method. Accuracy was calculated using the formulae-
Accuracy = (TN + TP) / (TN +TP+FN + FP)
TN=True Negative, TP=True Positive, FN=False Negative, FP= False Positive

Pearson's correlation coefficient, differences of mean & standard deviation between pair's results gave variability of Sahli's method for haemoglobin concentration. The intraclass correlation coefficient was analyzed to determine the within subject variability of the measured haemoglobin [12].

The Percentage bias haemoglobin concentration by Sahli's method was calculated as follows[13]:

$$\frac{\text{Sahli's Readings} - \text{HiCN Readings}}{\text{HiCN Reading}} \times 100$$

Result: There were 102 (59%) males and 71(41%) females within 18 to 23 years age group, out of 173 participants in the study.

Variability and reliability of results: Haemoglobin estimation by Sahli's method on capillary blood was within the range of 10 to 13.6gms/dl, with mean value of 11.8gms/dl. While haemiglobincyanide (HiCN) method estimated haemoglobin value within 9 to 14gms/dl, with 12.4gms/dl mean value in capillary blood. In venous blood, by Sahli's method mean haemoglobin concentration was 12gms/dl & was within 10 to 13gms/dl range. Also by HiCN method mean haemoglobin concentration was 13.1 gms/dl and within range of 8.3 gms/dl to 16gms/dl in venous blood. (Table No 2)
The descriptive statistics of the Haemoglobin concentration, Sahli's method had consistently lower mean values as compared to HiCN method in capillary and venous blood. There was positive correlation coefficient ($p<0.05$) between Sahli's method and HiCN method in capillary and venous blood. (Table N0 2)

As shown in table no 3, haemoglobin measured by Sahli's method estimated lower values compared to HiCN method with mean difference of 0.62 gms/dl(95% C.I : 0.51 to 0.73.gm/dl, $p<0.001$) in capillary blood and 1.1 gms/dl(95% Cl : 1.17 to 1.26, $p<0.01$) in venous blood sample in same subjects. Sahli's method had statistically significant ($p<0.05$) lower haemoglobin values in capillary blood compared to venous blood. Also HiCN level was lower in capillary blood than venous blood

with mean difference of 0.64 gms/dl (95% CI 0.45 to 0.83, p<0.01).

Table No 2: Descriptive statistics of measurement of haemoglobin by both methods in capillary blood and venous blood samples.

Haemoglobin	Sahli's Method		HiCN Method	
	C.B	V.B	C.B	VB
Mean (gms/dl)	11.8	12.4	12	13.1
S.D (gms/dl)	0.93	1.12	0.83	105
Normal (%) M>13 gms/dl F>12gms/dl	39.9	50.3	37	53.8
Mild anaemia (%) M=11to12.9gms/dl F=11to11.9gms/dl	52	43.3	59	42.2
Moderate (%) 8 to 10.9 gms/dl	8.1	6.4	4	4
Pearson's Correlation	0.75*		0.635*	

* Correlation is significant at the level of 0.01 level C.B-Capillary blood, V.B-Venous blood, M-male, F-females

Table No 3: Comparative indices of the methods of haemoglobin estimation.

Method	Paired Differences				value
	Mean	S.D	95% CI of the Difference		
			Lower	Upper	
S.M to HiCN.M on CB	-0.62	0.74	-0.73	-0.51	-10.8**
S.M to HiCN.M on VB	-1.1	1.17	-1.26	-0.92	-12.3**
S.M on CB to VB	-0.2	0.81	-0.29	-0.05	-2.7
HiCN.M on CB to VB	-0.64	1.3	-0.83	-0.45	-6.63**

**p value is significant at the 0.01 level. S.M= Sahli's Method, HiCN.M= Haemiglobincyanide method, CB=Capillary Blood, VB=Venous Blood.

The intraclass correlation coefficient (measures of consistency or agreement of values within cases) of Sahli's method compared to HiCN method was 0.849(95% CI 0.796 to 0.888) and 0.700(95% CI 0.596 to 0.778) in capillary and venous blood respectively. The lowest intraclass coefficient was in venous blood than capillary blood. The within subject variability of measured haemoglobin was more by venous blood in Sahli's method.

As shown in Table No 4, Haemoglobin concentration of capillary blood as well as venous blood samples gave more anaemic results by Sahli's method than HiCN method.

Table No 4: Diagnosis of Severity of anaemia according to haemoglobin cut-off level as per WHO.

Anaemia	S.M on CB	HiCN.M on CB	S.M on VB	HiCN.M on VB
No	69 (39.9%)	87 (50.3%)	64 (37%)	93 (53.8%)
Mild	90 (52%)	75 (43.3%)	102 (59%)	73(42.2%)
Moderate	14 (8.1%)	11 (6.4%)	7 (4%)	7 (4%)

Accuracy: Sahli's method compared to the HiCN method had higher sensitivity (90%) in venous blood compared to capillary blood (83.7%). Low specificity of anaemia detection was noted by Sahli's method in venous blood (60.2%) than capillary blood (63.2%). A high negative predictive value was noted in venous blood (87.5%) and false positive percentage was higher in capillary and venous of blood samples. The accuracy of Sahli's method in diagnosis of anaemia in capillary & venous blood was 73.4% & 74% respectively (Table 5).

The haemoglobin concentration by Sahli's method compared with HiCN method in capillary blood had mean bias of -4.7 %(S.D= 6.7%) while in venous blood of -7.6% (S.D=8.7%). Thus haemoglobin concentration in capillary blood had lower bias compared to venous blood by Sahli's method.

The bias of 10% in haemoglobin concentration was noted in 84% subjects in capillary blood and 56.3% in venous blood by Sahli's method. 11 to 20% bias was detected in 15% subjects by capillary blood and 39.1% by venous blood of haemoglobin concentration by Sahli's method.

Table No 5:
Reliability of Sahli's Method for haemoglobin estimation in capillary Blood (CB) and venous blood (VB) amples.

S.M	Sensitivity	Specificity	Accuracy	PPV	NPV	PFV	PFP
CB	83.7%	63.2%	73.4%	69.2%	79.7%	16.3%	36.8%
VB	90%	60.2%	74%	66.1%	87.5%	10.0%	39.8%

Discussion: Laboratory procedures need to be interpreted for test precision especially for evaluating significance of small changes. So all laboratory test are needed to be evaluated with respect to both accuracy and reliability.

Sahli's method had lower haemoglobin values than the haemiglobincyanide method irrespective of site of blood collection. Sahli's method underestimated the haemoglobin in capillary blood by 0.62 gms/dl and 1.12gms/dl in venous blood. Thus capillary blood gives lower concentration of haemoglobin suggesting the need for venous blood preference for accurate results. Similarly few studies also reported the under estimation of haemoglobin by Sahli's method.
In study by Kapil et al Sahli's method compared to Hemocue had underestimated haemoglobin concentration by 1.06gms/dl [(12)]. Natarajan S et al had also found that results of lower haemoglobin by 0.37gms/dl using venous blood and 0.386 gms/dl in capillary blood on comparing Sahli's method with the coulter auto analyzer.[9]

The differences in results are due to inbuilt errors of Sahli's method of haemoglobin estimation. HiCN method has an advantage to giving precise and reliable results of haemoglobin estimation in the blood.[6]

The variation in finger prick samples values may be probably due to roulex formation and inevitable haemodilution at the finger tip[(7)], also as Sahli's method is dependent on natural light the subjective colour comparison makes it is less accurate.

Capillary blood is less sensitive and more variable in Haemoglobin estimation than venous blood because of extracellular fluid causes dilution of components present in the blood. Moris S et al also found with-in subject variability when capillary blood from two hands was compared using portable hemoglobinometer system.[5]

Although the technicians were trained to avoid squeezing the fingers we suspect some contamination with extracellular fluid during capillary blood collection as found in many studies. This difference indicates the variation due to site of blood sample or the physiological difference in hematocrit of blood in circulation. The results are in agreement to the study states that site of blood sampling had made a difference in the haemoglobin measurements.[9]

The mean value of haemoglobin concentration and the detection of anaemia by Sahli's method were different from HiCN method. Sahli's method had acceptable sensitivity in detection of anaemic individuals, but low specificity and high rate of false positive results which may cause healthy individuals to be diagnosed as anaemic by haemoglobin concentration in capillary and venous blood.

The low positive predictive value by Sahli's method in capillary and venous blood indicates that only limited proportion of those diagnosed to be anaemic may truly have less haemoglobin levels.
Results of high sensitivity and low specificity results of Sahli's method in detection of anemia are in agreement with study by Barduagni on comparison with Hemocue method.[8]

The intraclass correlation in study by Kapil et al was 0.96 and mean difference was found to be the correlation factor of 1.06 which was recommended for haemoglobin values obtained by Sahli's method to have scientifically valid estimate of haemoglobin comparison to Hemocue method.[14] Comparison of haemoglobin estimated by Sahli's and HiCN method by finger prick and venepuncture method of blood collection showed highly significant differences. Similarly estimation of haemoglobin in the samples by two different groups of workers by Sahli's method showed significant difference while HiCN method had no significant difference.[6]

Full blood count contributed to the patient's clinical diagnosis in only 7.5% of the patients with normal haemoglobin in general practice. In 81.1% cases with normal Haemoglobin no abnormal features were found in any parameters of the blood count.[2]

Haemoglobin is measured more accurately by automated procedures as compared to spectrophotometric, with <1.0% error as compared to 1.0 – 2.0 % error (coefficients of variation).[10]

Though Sahli's method was performed with all precautions trying to minimise error, suggestions as per WHO guidelines [11] are needed to be followed for Improving test performance by improving its accuracy and reducing variability of the results.

Conclusion: Haemoglobin level in capillary blood was relatively lower than venous blood by either of the methods. Sahli's method had lower accuracy in haemoglobin measurement than hemiglobincyanide (HiCN) method in capillary and venous blood. Thus it may label healthy individuals as anaemic. In Sahli's method correction factor of +0.6gms/dl for capillary blood and +1.12gms/dl for venous blood samples. Sahli's method being simple, easy to use and with immediate results has the limited level of accuracy and considerable variability of the haemoglobin values.

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