Variability of Haemoglobin Estimation Methods in Capillary and Venous Blood of Adult Anemic Patients.

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Abstract: *Introduction:* Hemoglobin (Hb) assessments is the most reliable indicator widely used to screen individuals for anemia and to evaluate responses to nutritional interventions. Determination of hemoglobin concentration for anemia assessment is among the most frequently performed laboratory tests in inpatient and outpatient care. *Method:* Adult 78 clinically suspected anemic patients (42 non pregnant female and 36 male) had hemoglobin estimation done. The capillary and venous blood samples of same patients were tested by Haemiglobincyanide (HiCN) method using Drabkin's procedure and Sahli's method for haemoglobin. Drabkin's HiCN method in venous blood was considered as the standard reference method. *Result:* The mean hemoglobin concentration was found highest by Drabkin's HiCN method in venous blood i.e. 10.5 gm/dl. The coefficient of variation was lowest for the using venous blood in Drabkin's HiCN method i.e. 12.4% and highest for the Sahli's method of capillary blood i.e. 22.8%. Lower correlation coefficient was noted of reference method with the Sahli's capillary and venous blood. Statistically significant (p<0.001) difference in the proportion of patients grades of anemia was noted in between the two methods. *Conclusion:* Sahli's method had higher coefficient of variability and lower haemoglobin estimation in the capillary and venous blood compared to the reference method.

Keywords: haemoglobin, anemia haemiglobincyanide, Sahli's method, Drabkin's HiCN method.

Introduction:

Anemia is a condition in which the number of red blood cells and consequently their oxygen-carrying capacity is insufficient to meet the body's physiologic needs.[1]

The prevalence of anemia is an important health indicator and when the hemoglobin (Hb) concentration is used with other measurements of iron status, can provide information about the severity of iron deficiency. Estimates of the prevalence of anemia depend on the methods used for assessing Hb concentration and on the cut-off point applied which are different for different groups in a population. Hb assessments are the most reliable indicator widely used to screen individuals for anemia, to draw inferences about the iron status of populations and to evaluate responses to nutritional interventions.[1]

Determination of Hb concentration for anemia assessment is among the most frequently performed laboratory tests in inpatient and outpatient care.[3]

Hb concentration is either measured from venous blood samples with hematology analyzers or from capillary blood samples with handheld point-of-care devices. Several methods are available, each with a number of disadvantages related to cost and stability of reagents. The less sophisticated the device, the more easily we can respond in a sustained way to the needs of primary health care to screen for anemia in the absence of laboratory based haemoglobinometry. There are number of methods used for measuring Hb, most common one in peripheral health centers is the Sahli's method.[4]

Portable hemoglobinometer method with use of capillary blood samples are useful screening tool for anemia, but that unreliability poses a significant problem whenever individual-level estimates are required.[5,6]

Laboratory result variability caused from random errors in the testing process and finding bias that results from systematic errors in the testing process which are found to be contributes to result inaccuracy.

The reliability of the method needs to be confirmed as unreliability widens the distribution of Hb values and results in biases in estimates of prevalence of anemia and response to intervention for it. Also it is important to know the inherent variability and limitations of current Hb measurements based on the device used and the patient assessed. So the present study was carried out to determine the variability of results by Sahli's method compared to Drabkin's Haemiglobincyanide (HiCN) method in estimation of hemoglobin in laboratory diagnosis of

anemia patients.

Materials and Methods:
This study was conducted in March to August 2015 in Department of Physiology of Shri Bhausaheb Hire Government Medical College and Hospital, Dhule (Maharashtra), India. By selective sampling 78 adult anemic patients (42 non pregnant female and 36 male) ready to participate were identified clinically and were evaluated for the hemoglobin concentration by two methods. The blood for the test was collected from a finger prick and venous puncture, after obtaining written informed consents of the patients. Study was permitted by institutional ethical committee.

The Inclusion criteria were adult male and non pregnant females, absence of co-morbidity and chronic disorders. For Exclusion criteria, clinically symptomatic without hemoglobin deficiency and acute infection was decided.

For capillary blood sample collection we used painless sterile lancets. Ring finger of left hand was pricked under all aseptic precautions. The first drop of blood was discarded; blood was then collected for the both Sahli's and Drabkin's HiCN method using a Sahli's pipette. Also 3 ml venous blood was collected from left forearm was transferred and divided to glass tubes for assessment by the two methods. Same person after training, was operating the same device for the duration of the study (one laboratory technician each for Sahli's and Drabkin's HiCN method) and each individually recorded the haemoglobin measurement.

Haemoglobin Measurement
Sahli's Method[7]:
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. 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 were 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 gm/dl present on the haemoglobin tube.

Drabkin's Haemiglobincyanide (HiCN) method[7]:
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 were lysed and the released haemoglobin was converted to Haemiglobincyanide. 20µl of blood was added to 5 ml Drabkin's diluting fluid collected by automated dispenser. Reading was taken on photocalorimeter with green filter (540nm). Haemoglobin values were calculated by using the formulae-

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

* Concentration of Standard = 60 mg/100ml.

Anemia was defined as a haemoglobin level <12.0 gm/dl for non pregnant females and < 13 gm/dl for male, according to WHO-UNICEF the cut-off point indicative for anaemia. The Hb cut-off gradation for non pregnant females as mild (11 to 11.9 gm/dl), moderate (8 to 10.9 gm/dl) and severe (<8 gm/dl) and for males it was as mild (11 to 12.9 gm/dl), moderate (8 to 10.9 gm/dl) and severe (<8 gm/dl). [1]

Analysis:
The results obtained were analyzed using software-Statistical package for social science software version 16 (SPSS 16). Paired t test was used to compare values obtained by Sahli's method with Drabkin's HiCN method for capillary and venous blood. Variability within samples was calculated from duplicate measurements and Pearson correlation test of the methods with the Venous reading of Drabkin's HiCN method (Reference result). Systematic differences between the methods were assessed using samples from the same participant. Chi square test was used to find the relationship between the grades of anemia by the estimation methods. The p value less than 0.05 were considered statistically significant.

Results:
In the study, 42 non pregnant female and 36 male anemic patients (78) were evaluated. They were within 20 to 58 years age group with mean age of 31.2 years.
Intra-sample variability: The standard deviation (SD) for the difference between the first and the second measurement of the same sample using the Drabkin's HiCN method was 2.6 gm/l for venous and 2.0 gm/l for capillary blood, while for the Sahli's method the SD was 3.2 gm/l for venous and 2.4 gm/l for capillary blood.

Comparison of haemoglobin concentration: The mean hemoglobin concentration was found highest by Drabkin's HiCN method in venous blood .i.e. 10.5 gm/dl. The coefficient of variation was lowest for the using venous blood in Drabkin's HiCN method i.e. 12.4% and highest for the Sahli's method of capillary blood i.e. 22.8% as shown in Table 1.

The correlation between the Drabkin's HiCN method

assessment of venous blood and the Drabkin's HiCN method assessment of capillary blood was higher (0.978) than that between Drabkin's HiCN assessment of venous blood and the Sahli's method for capillary (0.718) and venous (0.638) blood samples. The correlation of haemoglobin concentration results of Drabkin's HiCN assessment of venous blood with other methods was statistically significant (<0.01) as shown in Table 1.

Table 1 also shows the higher percentage of anemic patients were labeled to be severe grade by Sahli's method also the percentage of those with mild was reduced in the Sahli's venous haemoglobin estimation . The capillary blood had graded more anemic patients to be in moderate anemia compared to the venous haemoglobin estimation by either of the method.

Drabkin's HiCN method assessment of capillary blood was significant lower by 0.3 gm/dl than its venous haemoglobin estimation (p<0.001). The Sahli's assessment of capillary blood was also found to be lower by 0.4 gm/dl than its venous haemoglobin estimation (p<0.05). Statistically no significant (p>0.05) mean difference of haemoglobin concentration was found in between the two methods either in capillary and venous blood. (Table 2)

The difference in gradation of the anemia according to WHO cut-off was found to be statistically significantly (p<0.001) different in the two methods for capillary and venous blood estimation. (Table 3)

Table No. 1: Comparison of methods for assessing haemoglobin (Hb) concentration in blood of anemia patients (n = 78)

Parameter	Haemiglobincyanide Method		Sahli's Method	
	Venous	Capillary	Venous	Capillary
Mean (gm/dl)	10.5	10.2	10.3	9.9
S.D of mean (gm/dl)	1.3	1.4	1.7	2.3
Range (gm/dl)	5.8-12.9	6-12.7	6-12.3	6-12.2
Coefficient of variation	12.4%	13.7%	16.5%	22.8%
Correlation coefficient	-	.978**	.638**	.718**
Grade of Anemia as per WHO cut off				
Mild	34.6%	26.9%	48.7%	32%
Moderate	59%	66.7%	42.3%	59%
Severe	6.4%	6.4%	9%	9%

** Correlation is significant at the 0.01 level (2-tailed).

Table No.2: Comparison of haemoglobin concentration in the methods in capillary and venous blood

Comparison	Mean difference of Hb	S.D	P value
Sahli's Venous vs. Capillary	0.4	1.3	0.042
Haemiglobincyanide Venous vs. Capillary	0.3	0.3	<0.001
Haemiglobincyanide Venous vs. Sahli's Venous	0.2	1.4	0.309
Haemiglobincyanide Capillary Vs Sahli's Capillary	0.3	1.0	0.075

Table No.3: Comparison of grades of anemia according to WHO cut-off level.

Comparison of methods	x^2 value	P value
Sahli's Capillary vs. Sahli's Venous	59.52	<0.001
Haemiglobincyanide Capillary vs. Venous	131.67	<0.001
Sahli's Capillary Vs Haemiglobincyanide Capillary	64.25	<0.001
Sahli's Venous vs. Haemiglobincyanide Venous	74.99	<0.001

Discussion:

Hemoglobin assessments are widely used to screen individuals for anemia and accordingly plan its management. Any assessment of laboratory result variability requires some form of repeated test measurements. So in our study the same patients sample were repeatedly analyzed by either of the methods with the specific site of blood collection.

For screening anemia it is important that the method should be rapid, easy to use, and inexpensive and also precise and accurate when compared with standard laboratory procedures.

The study derived that Hb estimation of capillary blood was lower than venous blood by either of the methods. So, Capillary blood is less sensitive and more variable in Hb estimation than venous blood because of extracellular fluid causes dilution of components present in the blood.

Patil et al. all found that Sahli's method had consistently

lower mean values as compared to Drabkin's HiCN method in capillary and venous blood.[5]

Morris S et al also found with-in subject variability of capillary blood from two hands when was compared using portable hemoglobinometer system. In capillary blood on the screening for anemia and diagnosis of low hemoglobin concentration had level of error of the range (CV from <3% to 6-7%).[6]

In our study it was noted that there is a high probability with more severity of anemia detected by the Sahli's method in venous and capillary blood and also lower haemoglobin estimation in capillary blood by either Sahli's and Haemiglobincyanide method.

Similar finding of lower haemoglobin in capillary blood was noted by Barduagn and colleagues as well Patil et al in their study stating the method often label healthy individuals as anemic.[4,5]

In our finding hemoglobin estimation by Drabkin's HiCN method of venous blood had higher correlation coefficient with that of the capillary blood and lower with Sahli's method. This finding are consistent with Morris et al who also noted reliability of correlation of result of the standard method with PHM was 69.3% as to our study we noted the lower correlation of 63.8% with Sahli's method in venous blood.[6]

Patil et al also noted positive correlation coefficient (p<0.05) between Sahli's method and Drabkin's HiCN method in capillary and venous blood.[5]

Direct cyanmethaemoglobin was found to have the correlation coefficient of 0.946 with measurement of capillary blood as noted in the comparative study by Sari et al.[8]

The present study showed that there is least variation in haemoglobin estimation in the Standard Haemiglobincyanide method while the variability was higher in the Sahli's method. The coefficients of variation were slightly higher than the estimated finding by the Morris et al in his study.[6]

It is stated that Sahli's methods remain a useful diagnostic tool to confirm the diagnosis of clinically suspected anaemia even in areas where the prevalence of anaemia is low and the haemoglobin level ranges from mild to moderate.[4]

The prevalence of anaemia was twice as high when the

indirect method was used (31-38%) and compared with the results of the direct method or the HemoCue (14-18%).[8]

This difference indicates the variation due to site of blood sample or the physiological difference in hematocrit of blood in circulation.

Similarly estimation of haemoglobin in the samples by two different groups of workers by Sahli's method showed significant difference while Drabkin's HiCN method had no significant difference.[9]

Reliability of hemoglobin assessments of capillary blood or within-subject fluctuations in hemoglobin concentrations, plausible that capillary blood would be more variable in hemoglobin concentration than venous blood because inclusion of extracellular fluid would decrease the concentration of components present in the red cell fraction, and the amount of extracellular fluid present in finger prick samples is likely to be very sensitive to the technician's handling of the patient's finger.[6]

Thus in low resource setting, Sahli's method that has its inherent variability of the haemoglobin estimation results needs to be taken into consideration in screening anemia and prognostic evaluation of the anemia management .

Conclusion:
Our study showed that in anemic patients there is least coefficient of variation in haemoglobin estimation in the haemiglobincyanide method by either venous or capillary blood. But the coefficient of variability was higher in the Sahli's method in capillary and venous blood. Sahli's method hemoglobin estimation had lower correlation coefficient in anemic condition.

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