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CLINICAL INVESTIGATIONS

INTRA-OPERATIVE POINT OF CARE HAEMOGLOBIN ESTIMATION: A COMPARISON OF THREE METHODS

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Introduction:
Perioperative blood transfusion is primarily dependent on the judgment of the anaesthesiologist. Estimation of blood loss is a difficult task and even with experienced clinicians it is found to be inaccurate. In several studies it has been found that the patients are exposed to the risk of unnecessary blood transfusion due to lack of proper indicator. Blood volume estimation along with haemoglobin estimation may be a better indicator for blood transfusion. Estimating the blood volume in a dynamic state during intraoperative period is difficult. Hence haemoglobin estimation can be considered as a marker for blood transfusion.

There are several methods available to determine haemoglobin concentration. During intraoperative period, the method has to be easy to perform, reliable and should be able to give quick results. Haemoglobin estimation with Sahli's acid hematin method is one of the techniques that can be used easily by the clinicians for immediate results. Inexpensive equipment and relatively easy technique makes this method popular but subjective variability of this colorimetric technique makes it less than optimum to consider as an ideal method. Coulter auto analysers are used in the clinical laboratories to estimate haemoglobin accurately and quickly. During intraoperative period this analyzer may not be available near the operating rooms and the need for the blood sample to be sent to the central location causes a delay in obtaining the results.

Hand held battery operated HemoCue® has been introduced in clinical practice as a device that gives results immediately and can be used in the operating room easily.

Considering the above reasons we have decided to undertake this study where we have assessed the reliability of haemoglobin estimation using Sahli's method and HemoCue® analyzer by comparing them with Coulter autoanalyser.

Coulter autoanalyser is the standard method that is available for haemoglobin estimation in our hospital. We have selected healthy clinically normovolaemic adults undergoing elective surgical procedures for this study. In order to find out the variability that can be present due to capillary and venous blood samples, we have taken both these samples while using Sahli's method and HemoCue® analyzer. These results are compared with the results obtained from the Coulter autoanalyser (lab method) using venous sample.

Methods:
The study was conducted after obtaining approval from the departmental dissertation committee. Fifty adult patients of ASA physical status 1 or 2 of either gender undergoing elective surgical procedures under general anesthesia participated in this study. Informed consent was obtained from all the patients.

Patients who were hypovolaemic or with rapid fluid shifts and with poor peripheral capillary circulation were excluded from the study.

All the patients were premedicated and kept nil per oral as per the standard protocol. On the day of surgery, standard monitors including non-invasive blood pressure, electrocardiogram and pulse oximeter were established. An infusion of maintenance intravenous fluids was started and anesthesia was administered (general or regional depending on the surgical need). There were two anesthesiology residents involved in the study as investigators. One investigator estimated haemoglobin using Sahli's method and the other estimated using HemoCue® analyzer. Same investigator estimated the Sahli's method using capillary and venous sample in all the 50 patients.
Under strict aseptic precautions capillary blood sample was obtained by pricking fingertip. Sample was obtained without squeezing the finger. Using this capillary blood sample the two investigators independently determined haemoglobin, one using Sahli's haemoglobinometer and the other using HemoCue® analyzer. Two milliliter of venous blood was obtained from the same limb and 1.5 ml of that blood was sent to the lab in an EDTA container for haemoglobin estimation using Coulter autoanalyser. The remaining venous blood was used to estimate haemoglobin by Sahli's method and HemoCue® by the two investigators. Both capillary and venous blood samples were obtained from the limb other than where the intravenous fluid was administered. There was no blood transfusion or blood loss during the sampling period.

Sahli's Method: The graduated tube placed between the brown glass standard of Sahli's haemoglobinometer is filled with N/10 hydrochloric acid up to lowest mark (mark 2). Blood sample obtained from the finger prick or from the vein is drawn into Sahli's pipette till 20 mm^{-3} mark and added into graduated tube containing N/10 hydrochloric acid. The blood and acid are mixed thoroughly with a glass stirrer and allowed to stand for 3 minutes for acid hematin to form. Distilled water is added drop by drop mixing it with a stirrer until color in the graduated tube is matched with the brown glass standard. Results were read as $g.dl^{-1.}$ present on the side of the graduated tube considering the lower level of meniscus.

HemoCue® method: The device is switched on. The blood is collected from the same prick site or from a vein into a microcuvette by capillary action avoiding air bubbles and is placed in a cuvette holder in the HemoCue®. The haemoglobin concentration will be displayed within 45 seconds as $g.dl^{-1}$

The results were tabulated and analysed using Pearson's correlation coefficient method and Bland and Altman statistical method for assessing agreement between the methods.

Results: Among the 50 patients who were studied, there were 29 male and 21 female patients. The youngest patient was 18 yrs old and the oldest was 70 yrs. There were no complications attributable to the study.

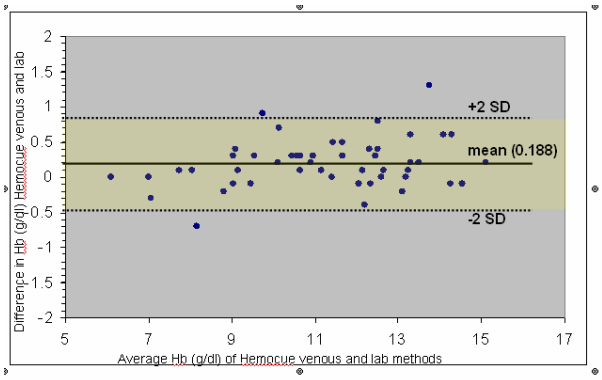
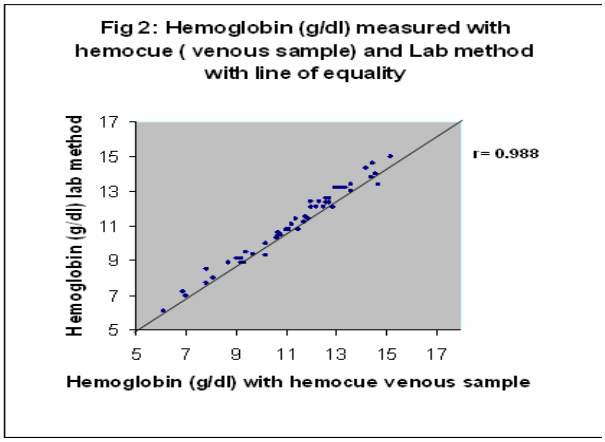


Fig 1 : Plot of the difference in Hb concentration (g/dl) using the hemocue venous And Lab methods against the mean of the two results showing the limits of agreement



The estimated haemoglobin values using Coulter auto analyser (lab method) ranged from 6.2 g/dl to 14.6 g/dl, with a mean value of 11.09 g/dl
The haemoglobin values obtained by HemoCue® analyzer using venous blood sample ranged from 6.1 g/dl to 15.2 g/dl with a mean of 11.28 g/dl. When compared with lab method, a correlation coefficient of 0.988 was obtained which is considered to be very good

The mean difference was found to be 0.188 g/dl with a standard deviation of 0.344 g/dl which means haemoglobin value estimated by Hemocue® analyzer using venous blood sample overestimated haemoglobin concentration by 0.188 g/dl as compared to lab method. The limits of agreement are defined as the mean difference ±2 SD and the calculated lower and upper limits (95% CI) for the study are – 0.500 g/dl and 0.876 g/dl respectively, which are again clinically insignificant. The 95% confidence interval for the lower limit of agreement is -0.6686 to -0.3314. For the upper limit of agreement the 95% confidence interval is 0.7014 to 1.0386. Both these suggest that the methods agree well with one another.

The haemoglobin values obtained by HemoCue® using capillary blood sample were in the range of 6.6 g/dl to 16.6 g/dl with a mean of 11.49 g/dl. When compared with haemoglobin values obtained by lab method a correlation coefficient of 0.931 was obtained. This is an acceptable coefficient. On further evaluation, the mean difference was 0.349 g/dl with a standard deviation of 0.837 g/dl. This suggests that when capillary blood was used, HemoCue® analyzer overestimated haemoglobin by 0.349 g/dl as compared to lab method. The lower and upper limits of agreement are -1.280 g/dl and +2.068 g/dl respectively.

The haemoglobin values obtained by Sahli's method using venous blood sample were in the range of 5.8 g/dl to 15.0 g/dl with a mean of 10.72 g/dl. A good correlation coefficient of 0.951 was obtained between these two methods.

Mean difference in haemoglobin concentration was -0.370 g/dl with a standard deviation of 0.660 g/dl. Sahli's method using venous blood sample underestimated haemoglobin by 0.370 g/dl as compared to lab method. The lower and upper limits of agreement for the study are -1.691 g/dl and 0.951 g/dl respectively.
The haemoglobin values obtained by Sahli's method using capillary blood sample were in a range of 6.4 g/dl to 16.0 g/dl with a mean of 10.71 g/dl. A correlation coefficient of 0.893 was obtained between the two methods.

Mean difference in haemoglobin concentration was 0.386 g/dl with a standard deviation of 0.987 g/dl. Sahli's method using capillary blood sample underestimated haemoglobin by 0.386 g/dl as compared to lab method. The lower and upper limits of agreement for the study are -2.36 g/dl and 1.588 g/dl respectively.

Discussion:
Intraoperative blood transfusion is common practice for anaesthesiologists. It is estimated that anaesthesiologists transfuse 50% of all blood products given to patients. The transfusion decision depends upon many unknown factors such as haemoglobin concentration at that time, the blood volume status, and an estimate of the patient's blood loss. A critical part of the decision of whether or not to transfuse blood to a patient should be based on an estimate of the patient's current haemoglobin concentration. Given the risks of transfusion of blood products, an accurate assessment of haemoglobin concentration is essential before any blood transfusion.[1]

In an ideal situation, transfusion would be based only on rapidly assessed laboratory measurements of haemoglobin concentration. In reality, however, it can take 30-45 minutes to get the results of a stat sample of blood analysed for haemoglobin concentration returned from the laboratory. In many intraoperative situations, decisions have to be made much sooner than later. We may have to depend on alternative methods of haemoglobin estimation like Sahli's method or HemoCue® method. In our study we have compared the haemoglobin concentration estimated by HemoCue® and Sahli's method against the lab method (Coulter auto analyzer).

The site of blood sampling can make a difference in the haemoglobin result[5, 11] Usually capillary blood sample is used with HemoCue® analyzer

and with Sahli's haemoglobinometer. During laboratory analysis venous blood sample is used. In general, obtaining the capillary blood sample is convenient compared to venous sample and the small variation may not be of great significance. The situation in the operating room can be different in that it is easier to obtain venous sample and accurate estimation can be critical. In our study we compared the haemoglobin values using capillary blood sample and venous blood sample with commonly used Sahli's haemoglobinometer and the more recent HemoCue® analyzer against the coulter auto analyser (lab method) using venous blood sample.

In our study haemoglobin estimated by HemoCue® using venous sample closely correlated with lab method. There was a small difference of 0.19 g/dl with standard deviation of 0.34. HemoCue® analyzer on an average overestimates haemoglobin by 0.19 g/dl and the range is 0.5 g/dl lower to 0.87 g/dl higher than lab method. These two methods showed a good correlation co-efficient of 0.988. We consider this as clinically acceptable range since a difference of less than 1.0 g/dl is not significant to affect the transfusion decision. When we used capillary blood sample with HemoCue® analyzer the mean difference was 0.39 g/dl with standard deviation of 0.837. Thus with capillary blood sample, HemoCue® analyzer can overestimate haemoglobin upto 2.06 g/dl or it can underestimate up to 1.28 g/dl.

Even though the correlation coefficient between HemoCue® capillary blood sample and lab method was 0.931, the limits of agreement were between -1.28 g/dl and 2.06 g/dl. That means HemoCue® analyzer estimated haemoglobin by 1.2 g/dl lower or 2.06 g/dl higher than lab method. This wide range of variability is difficult to accept.

When Sahli's method using venous and capillary blood sample was evaluated by comparing with lab method it was found that Sahli's method underestimated haemoglobin by 0.37 g/dl in venous blood sample and 0.38 g/dl in capillary blood sample. Also when Sahli's was compared with HemoCue® using both venous and capillary blood sample, it was found that Sahli's method underestimated haemoglobin by 0.56 g/dl in venous blood sample and by 0.74 g/dl using capillary blood sample.

The difference obtained between capillary and venous sample may be due to sampling site error or due to real differences in hematocrit in venous and capillary circulation. Capillary fluid shifts during the course of major surgeries could theoretically alter these findings temporarily. Capillary samples analysed in the lab would be the only way to resolve the issue fully. Though capillary samples still gives a good estimate of the patients haemoglobin concentration with minimal invasiveness the potential for up to 2.06 g/dl error with HemoCue® method suggests that samples from venous blood are preferred when we need to know more accurate results.

Kapil et al compared HemoCue® analyzer with Sahli's method and they found that Sahli's method underestimates haemoglobin by 1.06 g/dl compared to HemoCue® analyzer[6]. Sahli's method has been known for several limitations. The results are subjective and less accurate, being dependent on natural light and observer perception of colour. These might be possible reasons for lower assessment of haemoglobin values by Sahli's method as compared to standard lab method or HemoCue® analyzer.

Schench et al evaluated Hemocue® by comparing with cyanmethaemoglobin method using Hemalog according to ICSH and found a good correlation (r- 0.96).[4] Also they analysed Hemocue® for influence of lipid particles on turbidity errors and found Hemocue® insensitive for errors of haemoglobin due to admixture of lipid particles 3.8% v/v.

Neville evaluated Hemocue® in two settings. Hospital laboratory operated by trained lab staff and a general practice health operated by nurse. There was a good correlation (r = 0.99) in lab conditions where as the correlation of results when used by practice nurses in health centers was 0.61, which was poor. They concluded poor mixing of full blood count sample before analysis was the reason for poor correlation.

Lardi et al compared Hemocue® with Coulter Max M® for measuring haemoglobin concentration[8].

They analysed 52 arterial blood samples obtained from 13 patients during aortic surgery. There was no significant difference between the results with a mean of 10.94g.dl^{-1} and 10.90g.dl^{-1} for Hemocue and Coulter respectively. The limits of agreement of two methods were -0.37 and 0.45g.dl^{-1}. They concluded that with adequate training, Hemocue provides comparable haemoglobin results.

McNulty et al in there study on 25 samples concluded that Coulter measurement for all in vivo blood samples was provided by bedside photometry (Hemocue®)[9].

Our study included adult patients posted for elective surgical procedures with a haemoglobin concentrations ranging from 6.1 g/dl to 14.6 g/dl. (measured by coulter analyzer). We have not compared in extremes of haemoglobin values. The HemoCue® manufacturers claim that the instrument has a measuring range of 0 to 25.6 g/dl. The accuracy of HemoCue® analyzer in extremes of haemoglobin values requires further evaluation. Use of HemoCue® in assessing the blood loss in TURP surgeries by analyzing the haemoglobin in the returning irrigation fluid can also be studied.

To conclude, the haemoglobin determination by HemoCue® combines the convenience of point of care testing with accuracy and ease. The venous blood sample is preferred over capillary blood sample while using HemoCue® analyzer. Sahli's method can also be used for point of care estimation when HemoCue® is not available provided a correction factor (underestimation) of 0.57 g/dl for venous blood sample and 0.78 g/dl for capillary samples are taken into consideration.
To conclude.

1. HemoCue® analyzer provides comparable results with Coulter auto analyzer (lab method) when venous blood samples are used for haemoglobin estimation
2. HemoCue® analyzer using capillary blood samples overestimates haemoglobin when compared to Coulter auto analyser
3. Sahli's method using both venous and capillary blood samples underestimates the haemoglobin when compared to Coulter auto analyser.

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