Comparison of efficacy of filter paper cyanmethemoglobin method with automated hematology analyzer for estimation of hemoglobin

Lalit Kumar, Ranjit Kangle

Abstract:

BACKGROUND: Screening of hemoglobin (Hb) before blood donation is one among the vital tests. It is performed to select a blood donor to prevent the collection of blood from an anemic person. However, no accurate, cost-effective, reliable, and standardized method is available to estimate Hb.

OBJECTIVE: The aim is to evaluate the efficacy of filter paper cyanmethemoglobin (FPCH) method with the automated hematology analyzer in the estimation of Hb concentration for screening of a suitable donor.

METHODOLOGY: This was a cross-sectional study in which the blood samples of 2000 patients visiting KLE’s Dr. Prabhakar Kore Charitable Hospital, Belagavi, were collected in vials and directly estimated for Hb using automated hematology analyzer. To evaluate the efficacy of FPCH, 20 μL of blood sample was transferred onto Whatman filter paper and dried at room temperature. After drying, it was placed in 5 mL of Drabkin’s solution for 30 min. Optical density was estimated by measuring the absorbance. Data were analyzed using SPSS version 20. The correlation coefficient, paired t-test, and difference between the means of both the methods were calculated.

RESULTS: The mean Hb estimated by FPCH was 11.25 g/dL and automated hematology analyzer gave 11.35 g/dL. The difference in the means of both the methods was 0.1 g/dL. Paired t-test was done to test the level of significance and the result was 8.151 (95% confidence interval: 0.08–0.13 g/dL, P < 0.001). The correlation coefficient was found to be 0.976 (P < 0.001).

CONCLUSION: FPCH is an efficient method, which is comparable to the automated hematology analyzers for Hb estimation. It could be used as an alternative screening tool for detection of Hb in a blood donation camp.

Keywords: Automated hematology analyzer, filter paper cyanmethemoglobin, hemoglobin

Introduction

Hemoglobin (Hb) is a two-way, iron-containing, and oxygen-transporting carrier protein found in the red blood cells (RBCs). It is made up of four connected globin chains: two alpha- and two beta-globin chains. Porphyrin is an iron-containing heme compound, which is vital for the transportation of gasses. Hb helps in transportation of oxygen from lungs to the tissues and carbon dioxide from tissues to the lungs. Carbon dioxide binds to the globin protein as carbaminohemoglobin. It maintains the shape of the RBCs; if the Hb structure is abnormal, it can interfere with the flow and function of RBCs. It constitutes approximately 90% of the RBC’s dry weight.

Anemia is the deficiency of Hb in the RBC. According to a survey conducted by the

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World Health Organization (WHO) in 93 countries around the world, 1.62 billion people have been affected from anemia. Iron deficiency anemia (IDA) is the most common cause of anemia. It is a serious health problem, as it affects the development, work performance, and behavior of an individual.[4‑6] IDA can be treated by oral supplementation of iron, which has proved to improve the overall status of health.[7] Therefore, it is vital to estimate Hb to eliminate anemia. However, due to lack of proper standard methods for estimating Hb in the rural and some inaccessible areas in developing countries, multitudes of people are suffering from anemia.[8]

A simple, cost-effective yet reliable, and accurate method to determine Hb level is the need of the hour. Numerous methods and devices based on different principles are available such as Sahli’s acid hematin method, hemoglobin color scale (HCS), filter paper cyanmethemoglobin (FPCH), and automated hematology analyzer.[8,9] In our study, the efficacy of FPCH method and automated cell counter using sodium lauryl sulfate (SLS) method is compared to estimate Hb levels in terms of accuracy, cost, and reliability.

### Methodology

This 12-month, cross-sectional study was conducted at KLE’s Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi, between January 2014 and December 2014. A total of 2000 patients were studied after obtaining their consent. Patients undergoing routine blood investigation at the Department of Pathology of KLE’s Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi, were included in the study. Clotted and hemolyzed blood samples were excluded from the study. Ethical clearance was obtained before the commencement of study from the Institutional Ethics Committee of Human Subjects Research, Jawaharlal Nehru Medical College, Belagavi.

The blood (2 mL) was collected from all the study participants following aseptic precautions in an ethylenediaminetetraacetic acid (EDTA) Vacutainer tube from the patients. To eliminate investigator bias, single-blinding was done and samples were coded using random six digit code. The Hb level of the coded sample was estimated using the five-part automated hematology analyzer, which undergoes daily quality control tests. For FPCH method, the blood sample (20 μL) was transferred onto Whatman filter paper and dried at room temperature. The dried samples were individually placed in 5 mL of Drabkin’s solution in a test tube for 30 min. The optical density (OD) is read using a digital colorimeter at an absorbance wavelength of 540 nm. It was calibrated to zero using distilled water. First, the standard for the cyanmethemoglobin was estimated and recorded. After 30 min, the OD of the samples was recorded. Hb values for indirect cyanmethemoglobin method were calculated using the formula:

\[
Hb \text{ (g/L)} = \frac{A_{540} \text{ of test sample}}{A_{540} \text{ of standard}} \times \frac{\text{concentration of standard} \times \text{Dilution factor(251)}}{1000}
\]

Where,

- \(A_{540}\) of test sample: absorbance of the test sample at 540 nm.
- \(A_{540}\) of standard: absorbance of the Hb standard at 540 nm.
- Concentration of standard: concentration of hemoglobin cyanide (HiCN) in the solution; it is taken as 60 mg/dL.
- Dilution factor was taken as 251 (20 μL of blood plus 5 mL of reagent).[10]

All the data obtained were tabulated on Microsoft Excel spreadsheet and analyzed using SPSS version 20 (SPSS, Inc., Chicago, IL). The correlation coefficient between the two methods was calculated and paired t-test was used to determine the difference between the mean values of the two methods.

### Results

A total of 2000 patients were studied for 12 months. Patients were from all walks of life with age varying from day 1 infant to 92 years adult. The majority of the patients were between the age of 11 and 60 years (75.7%). Out of 2000 patients, 965 (48.25%) were women and 1035 (51.75%) were men.

According to the WHO recommendation for classification of anemia in various age-groups, the patients were allotted different subgroups according to their age and Hb values. On the basis of age, patients were divided into age-groups such as 6–59 months, 5–11 years, and 12–14 years. Patients aged more than 15 years were subdivided into groups of men, women, and pregnant women. Furthermore, based on the Hb values estimated by automated hematology analyzer and FPCH, each group was further divided into normal, mild, moderate, and severe anemia as per the recommendations of the WHO.[11]

The outcome of this study suggests that 66.7%, 56.1%, and 64.7% of children between the age of 6–59 months; 5–11 years; and 12–14 years of age were anemic,
respectively. Among the adults, 62% of men, 67% of women, and 67% of nonpregnant women were found to be moderately anemic. The mean Hb value estimated for FPCH and automated hematology analyzer was 11.25 g/dL (standard deviation [SD] ±2.55 g/dL) and 11.35 g/dL (SD ± 2.51 g/dL), respectively. The difference in the means of both the methods was 0.1 g/dL. This signifies that values obtained from the FPCH method are equivalent to the value provided by hematology analyzer. The level of significance was tested by paired t-test and the result was 8.151 (95% confidence interval: 0.08–0.13 g/dL, P < 0.001). Correlation coefficient was found to be 0.976 [P < 0.001; Table 1 and Figure 1]. The percentage of the difference between the Hb values of both the methods was calculated. The majority of the results showed a variation of ±10.0%, signifying good correlation between the two methods.

Table 1: Comparison of filter paper cyanmethemoglobin method and automated hematology analyzer for hemoglobin estimation

|                      | FPCH method | Automated analyzer |
|----------------------|-------------|--------------------|
| Mean Hb (g/dL)±SD    | 11.25±2.55  | 11.35±2.51         |
| Conclusions          | P           |                    |
| Differences in means (g/dL) | 0.10      |                    |
| Paired t-test        | 8.15 (95% CI: 0.08–0.13) | <0.001 |
| Pearson’s correlation coefficient (r) | 0.976 | <0.001 |

SD = Standard deviation, CI = Confidence interval, FPCH = Filter paper cyanmethemoglobin, Hb = Hemoglobin

**Discussion**

In India, anemia is a public health concern, which affects mainly preschool children and women of reproductive age. Low level of Hb is an apprehension among the young children, as it could result in impaired cognitive performance, motor development, and make them susceptible to infections. In pregnant women, anemia can result in premature delivery, prenatal death, and low birth weight.\[12\]

The present study was conducted to compare the Hb values estimated by FPCH method with the values assessed by the current reference standard, that is, automated hematology analyzer that utilizes SLS method and also to estimate the prevalence of anemia in the study population. According to the study done in the Department of Virology of Hospital for Children NHS Trust, London, authors stated that the dried blood spots could also be used as a medium for various investigations such as testing for human immunodeficiency virus, human T-lymphotropic virus 1, hepatitis C virus, and *Toxoplasma gondii*.\[13\] We have used the dried blood spots to effectively transfer the blood samples in a labeled bag and used the samples on a later date to estimate Hb values of the same sample. The estimated Hb values derived from the said samples on filter paper were comparable to the gold standard used in the study. As the authors of the above article mentioned, the use of dried blood spots lead to reduction of risks.\[13\]

Multiple studies have been conducted in India using FPCH method to estimate the Hb values and in the process detect anemia in the population. A study conducted by Aggarwal *et al.* to test the validity of anemia by palmar pallor used the FPCH method as a reference standard to detect the levels of Hb.\[14\] Two studies conducted in rural Wardha, India, proved the utility of FPCH method for the detection of anemia in rural setup.\[15,16\] Toteja *et al.* conducted a study in several states of India to detect the prevalence of anemia in pregnant women and adolescent girls.\[17\]

FPCH method makes it convenient and easy to collect samples in any condition. With the use of lancets to draw the blood and storage on a filter paper, the need to carry syringes and tubes is eliminated. Furthermore, the need to carry a temperature-controlled storage container for the EDTA tubes with samples is not required. Hb estimation by indirect cyanmethemoglobin has high sensitivity and specificity of 76%, as compared to the commonly used method for estimation of Hb in rural areas, that is, HCS method that has a lower sensitivity and specificity of approximately 50%. A small variation is noticed between capillary and venous samples; while sensitivity to detect anemia remains the same in both, specificity is mildly reduced in capillary samples. The coefficient of variation for FPCH method is 11.38%.\[9\]

Shah *et al.* compared Hb estimation by a noncyanide method with HiCN method. They found out an excellent correlation (r = 0.98, P < 0.001) between the noncyanide...
method and HiCN method. In our study, we compared HiCN method with automated hematology analyzer, which utilized a noncyanide method for determination of Hb. We also found similar correlations ($r = 0.98$, $P < 0.001$).

Another study published in the Journal of Clinical and Diagnostic Research by Chakravarthy et al., estimated Hb values in 2000 patients using HiCN method with Drabkin’s reagent, and compared it to SLS reagent. Their patients’ age ranged from day 1 to 88 years, whereas Hb levels ranged from 3.8 g/dL to 20 g/dL. The Pearson’s correlation coefficient was reported as 0.98 with $P < 0.001$. We also studied Hb values in 2000 patients for 1 year and their age ranged from newborns to 92 years. The Hb values varied from 2.0 to 20.4 g/dL. The Pearson’s correlation coefficient was calculated and it matched with the above mentioned study. The “$r$” value was 0.98 with $P < 0.001$, which signifies excellent correlation.

In the year 2000, Azim et al. compared cyanmethemoglobin method and automated cell counter for accuracy, cost-effectiveness, suitability, and feasibility. They found that both methods were accurate and precise, but FPCH method was comparatively cheaper. The cost for Hb estimation using automated analyzer was Rs. 50/test, whereas cost for FPCH method was just Rs. 3.5/test. Hb level was increased by 0.85% when measured by cyanmethemoglobin method compared to automated analyzer. The difference in the mean Hb of both the tests was 0.1 g/dL and coefficient of variation was 2.7% as compared to controls for cyanmethemoglobin method. Maintenance of automated analyzer caused frequent problems, whereas FPCH method required negligible maintenance. In our study, we had similar conclusions. The mean Hb by FPCH method and automated hematology analyzer was $11.25 \pm 2.55$ g/dL and $11.35 \pm 2.51$ g/dL, respectively. The difference in mean Hb was 0.1 g/dL. However, in our study, the Hb value was decreased by 0.88% when measured by FPCH method as compared to the automated hematology analyzer. Furthermore, the cost for Hb estimation using automated analyzer was Rs. 50/test, whereas cost for FPCH method was just Rs. 10/test.

FPCH method costs lesser than HemoCue, which costs Rs. 35/test. Few studies conducted in India show that HemoCue overestimates the Hb values by about 2 g/dL. The hot and humid climate and duration of opening of microcuvette box also affects the Hb values estimated by HemoCue. Copper sulfate method is also another cheap and commonly used alternative to HemoCue for detection of Hb. A study done by Samuel Antwi-Baffour shows that using copper sulfate method underestimates the Hb, leading to increase in deferral of prospective blood donors.

All the data obtained highlight that statistically strong significant correlation exists between the Hb values estimated by FPCH method and automated hematology analyzer.

**Conclusion**

FPCH is an efficient method, which is comparable to the automated hematology analyzers for Hb estimation. The FPCH method along with dried blood spots could be used in mass population-based surveys and for mass screening of blood donors before conducting blood donation camps. It could be a valuable asset in a resource-poor setting of a blood donation camp as the results obtained are reliable, reproducible, and cost-effective.

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**Conflicts of interest**

There are no conflicts of interest.

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