Methods for Hemoglobin Estimation: A Review of “What Works”

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Abstract

Anemia is widely prevalent in developing world and is a public health challenge in India. Hb assessment is a reliable indicator for anemia screening. However, there are multiple techniques with different applications available for Hb estimation. The present work was undertaken to review Hb assessment methods that can work best from the developing country perspective. We reviewed published literature through the PubMed database for studies comparing different techniques of Hb estimation. A search for reports on prevalence of anemia both at the global and national level was also undertaken. A total of 74 articles were included in this review. It was realized that even though there are multiple techniques for Hb estimation, a method which is quick, valid and reliable is needed to detect anemia. Direct cyanmethemoglobin method has been the gold standard for hemoglobin estimation but other methods like hemoglobin color scale, Sahli technique, Lovibond-Drabkin technique, Tallqvist technique, copper-sulfate method, HemoCue and automated haematology analyzers are also available. Each method has a different working principle and its own advantages and disadvantages. Despite conflicting reports, it has been observed that HemoCue is the method of choice for initial screening of anemia because it is reliable, portable, does not require power supply and easy to use in poor resource settings without requiring extensive training of health workers. Hemoglobin color scale developed by HCS is another potential method that can be used in field situations. However, such methods should be further investigated through larger studies before actual implementation.

ABBREVIATIONS

WHO: World Health Organization; DLHS: District Level Health Survey; NFHS: National Family Health Survey; Hb: Hemoglobin; HCS: Hemoglobin Color Scale; HiCN: Direct Cynmethemoglobin; HLL: Hindustan Lifecare Limited; OPD: Outpatient department; AIIMS: All India Institute of Medical Sciences;

INTRODUCTION

Anemia is widely prevalent in the developing world and a major public health problem needing urgent attention of policy planners. WHO report of 1992 stated an overall prevalence of 42% of anemia in women of developing countries; 51% in pregnant women and 41% in non-pregnant women. Out of these nearly half the total numbers of women are from South East Asia [1]. Later in 1993-2005, WHO database on global prevalence of anemia reported an overall prevalence of 25% where 65.5% of pre-school children, 48.2% of pregnant women and 45.7% of non-pregnant women were estimated to have anemia [2]. A recent systematic analysis of global anemia burden has reported that the prevalence of anemia has decreased from 40.2% in 1990 to 32.9% in 2010. Also, females had higher prevalence and mean severity of anemia in virtually all regions and throughout adulthood whereas male children had higher anemia prevalence than females [3].

Anemia is a public health challenge in India and mostly affects women of reproductive age and preschool children. The District Level Health Survey (DLHS) 2002-2004 reports an extremely high burden of anemia with 80% of adolescent girls and around 93% of pregnant women suffering from this condition [4]. The National Family Health Survey (NFHS)-3rd in 2005-2006 reported that 55% of women and 24% of men were also found to have hemoglobin levels lower than the cut off [5]. Studies have reported an increasing trend in prevalence of anemia from 49.7% (NFHS-2, 1998-1999) to 50.5% (NFHS-3, 2005-2006) [6]. Moreover, the severity of anemia was higher in pregnant population [7]. A survey of 16 districts from 11 states of India reported 90% prevalence of anemia in adolescent girls and 84.9% in pregnant women [8]. A similar prevalence of 90% in
girls has also been reported from a survey of 35 states and union territories of India [9]. Despite several interventions through national programmes, anemia is still endemic in women. Low level of hemoglobin among pregnant women results in premature delivery, low birth weight and prenatatal mortality. It also retards physiological growth in adolescent girls [4].

Anemia in young children is a serious concern because it can result in impaired cognitive performance, behavioural and motor development, coordination, language development, and scholastic achievement as well as increased morbidity from infectious diseases [4,5]. One of the most vulnerable groups is children in the age group of 6-23 months [5]. DLHS-2 reported that more than 96% of children are suffering from anemia whereas NFHS-3 reported that 70% of children in the age group of 6-59 months are anemic [4, 5]. NFHS-3 also observed that even for mothers who were not anemic, 62% had anemic children (6-59 months). The prevalence of anemia in children rose steadily with the mother’s level of anemia, reaching 82% for children of mothers who were severely anemic [5]. A recent study published in 2014 found 69.6% prevalence of anemia in children (6-30 months) [10]. Therefore, more than half of the population is suffering from anemia.

**METHODOLOGY**

We conducted an online search of PubMed using terms “hemoglobin OR haemoglobin”, “prevalence of anemia OR anaemia”, “hemoglobin estimation”, “HemoCue”, “automated haematology analyzer”, “cyanmethemoglobin”, “non-invasive hemoglobin estimation” and a combination of these. We considered studies comparing commonly used methods for hemoglobin estimation in any setting, reporting validation and/or reliability for further review. The search was limited by species (humans) and full text availability; only articles published in English were considered. We also searched reports on prevalence of anemia in India and worldwide. The reference list of identified articles was also reviewed and the articles considered relevant were selected.

**RESULTS**

A quick, valid and reliable estimation of hemoglobin status is vital to detect anemia in the population at risk. An early diagnosis of anemia can provide an opportunity to remedy the situation in the public health problem context as well as prevent progression of the condition (Figure 1).

The measurement of Hb has traditionally relied on the services of a well-equipped clinical laboratory. Direct cyanmethemoglobin method has been the gold standard for hemoglobin estimation and is cheap but time consuming. A number of other methods are available such as hemoglobin color scale, Sahli technique, Lovibond-Drabkin technique, Tallqvist technique, copper-sulfate method, HemoCue and automated haematology analyzers. Each method has a different working principle and its own advantages and disadvantages. Simple techniques to measure Hb exist but they are relatively expensive and require commercial reagents and good technical skills to interpret.

A number of methods with their specific working principle, advantages and disadvantages are present however most of these methods are no longer used. Haldane method for hemoglobin estimation is one of the oldest methods, based on carbon monoxide (CO) carrying ability of blood and comparison with a standard. However, commercially available standards are unreliable and fade quickly. Also, the method requires a source of CO [11]. Dare method uses a small glass chamber which is filled with whole blood by capillary action. The chamber is illuminated by a battery lit bulb and the color of blood is matched with a standard after viewing through an eyepiece [12,13]. Lovibond-Drabkin technique measures cyanmethemoglobin wherein the blood mixes with a solution and after standing time of 3 minutes the color of blood is matched with a color standard on discs from which the hemoglobin concentration is interpolated. The method was shown to outperform other comparators in a study conducted way back in 1983 [14]. The discs used are costly and the estimate is prone to error due to subjective interpretation of the color.

In another method known as Tallqvist, a drop of blood is placed on a strip of blotting paper and the concentration of hemoglobin is interpolated by comparing the color of the blood with a set of color standards on paper but the matching of standards and sample is extremely difficult [13]. A.O. Spencer method manufactured by an American company uses a drop of whole blood which is placed on one-half of a glass chamber and agitated with a small stick coated with saponin. The chamber is completed by a cover-glass held firmly by a clip and inserted into a slot in the instrument. Transmitted green light from a battery-lit bulb is compared with a variable standard on a split screen and the hemoglobin level and reads as g/100 ml of blood [12,13].

Copper sulphate method commonly used to ascertain Hb for blood donation is based on specific gravity of blood. A blood droplet is allowed to fall into copper sulphate solution of a specific gravity equivalent to that of blood with hemoglobin.
content of 100g/l and the movement of droplet is observed. The test is repeated with hemoglobin content of 80g/l. The samples are then categorized as below 80g/l, between 80-100g/l or over 100g/l [12]. A study published in 2007, evaluated the impact of CuS04 method on donor deferral and concluded that the CuS04 method does not give quantitative results, has a subjective end point and is inaccurate [15].

The cyanmethemoglobin method works on the principle of conversion of hemoglobin to cyanmethemoglobin by the addition of potassium cyanide and ferricyanide whose absorbance is measured at 540 nm in a photoelectric calorimeter against a standard solution [16]. In India approximately 70% of laboratories still use direct cyanmethemoglobin method (HiCN) for hemoglobin estimation especially in rural areas. There is a need to have non-cyanide methods to avoid environmental pollution by cyanide reagents. A study was conducted in a tertiary care hospital in Mumbai which compared cyanide method (HiCN) with non-cyanide methods (alkaline hematin method and alkaline borate method). The results showed excellent correlation among the three methods. The cyanide free methods also have advantages from safety standpoint as well as cost when compared to the standard HiCN method [17].

Another disadvantage of HiCN method is presence of turbidity which results in an inaccurate estimate when absorbance is measured at single wavelength of 540nm. HemoCue hemoglobin photometer now widely used compensates for this. The system consists of disposable microcuvettes containing dry reagents and a photometer. Blood is placed in the microcuvette which reacts with sodium deoxycholate, releasing hemoglobin by hemolysing erythrocytes. Then sodium nitrite converts hemoglobin to methemoglobin which, together with sodium azide, gives azidemethemoglobin. The absorbance is measured at two wavelengths (565nm and 880nm) in order to compensate for turbidity [18,19].

HemoCue is yet another method for estimation of Hb. A study carried out in blood donors found the sensitivity and specificity of HemoCue to be 94.1% and 95.2% as compared to 90.1% and 94.2% for direct cyanmethemoglobin method [20]. Specificity of HemoCue assessment of capillary (95.2%) or venous (94.2%) blood was found to be higher as compared to the direct (94.2%) or indirect (68.3% for capillary and 76% for venous blood) cyanmethemoglobin method by another study conducted in Indonesia. This study also concluded that in contrast to the other two methods, HemoCue gave the same results for capillary and venous blood [21].

Hemoglobin is also routinely measured using automated haematology analyzers which are reliable and accurate but expensive. This system is an automated blood cell counter which measures hemoglobin using non-cyanide method. A cross-sectional study carried out among pregnant women in Sudan found that hemoglobin concentration measured through HemoCue has lower levels of precision as compared to the automated analyzer [18]. On the contrary, another study concluded that comparison of hemoglobin estimation performed by lab personnel's showed an excellent correlation of 0.99 between HemoCue and automated analyzer. But in the same study, the correlation (0.61) result was unsatisfactory when performed by nurses in general practice, apparently because of operator error [22]. A hospital based study in Ghana, found no significant difference in hemoglobin concentration determined by HemoCue compared to cyanmethemoglobin and HemoCue compared to automated analyzer [16]. A study on pregnant women in Nigeria showed a positive correlation between HemoCue and cyanmethemoglobin method, recommending its use in rural areas [23].

A new non-invasive hemoglobin estimation instrument has been introduced, NBM-200. In this instrument a sensor is placed on the thumb and optical measurements are taken after temporary blood flow occlusion [24]. A study conducted in Korea compared NBM-200 and HemoCue with automated analyzer as a reference to detect percentage of ineligible blood donors who were correctly identified as ineligible to donate blood (sensitivity). The correlation between auto-analyzer and NBM-200 was 0.69 while that between auto-analyzer and HemoCue was 0.86. The sensitivity and specificity of NBM-200 was found to be 38.6% and 93.6% respectively, while for HemoCue sensitivity was 42.7% and specificity was 98.6%. However, the authors have mentioned that based on their findings this non-invasive instrument should be used as a screening tool with caution [24].

In 1995, WHO carried out studies for the use of hemoglobin color scale (HCS) to detect anemia. This is based on the principle that the colour of a drop of blood could reliably indicate anemia. The colour would be matched against predetermined shades of red. The scale comprises of a small card with six shades of red that represent hemoglobin levels at 4, 6, 8, 10, 12 and 14g/dl respectively. A drop of blood is placed on the strip provided and after 30 seconds the color of blood is matched against the shades on the scale [25]. The colour technology and test paper strip available determines the accuracy of the method. With the available technology to perfect the material on which blood can be absorbed and computerized spectrophotometric analysis to identify colours, the method has undergone changes.

Initial results indicate that the scale was found to have a sensitivity of 95% and specificity of 99.6%. However, a study was recently carried out in All India Institute of Medical Sciences, New Delhi to compare WHO developed HCS (COPAC) with Sahli’s technique which is the method recommended by the Government of India for hemoglobin estimation and automated analyzer. The study concluded that Sahli and auto-analyzers are in good agreements but HCS is not efficacious (30% sensitivity) and should not be used for hemoglobin estimation [26].

Another HCS has been developed by HLL (Hindustan Lifecare Limited), India to screen anaemia in community settings that includes six color shades of Hb concentration on a paper-card, at interval of two units of Hb measurement and special adsorbent test-strips. It is a simple device for estimating Hb. By matching the color of a drop of blood on a test-strip with the shades on the paper-card, an estimate of the Hb concentration can be arrived upon. A small pilot carried out at AIIMS gave encouraging results (Personal communication. Saxena R. Department of Hematology. All India Institute of Medical Sciences, New Delhi).

Preliminary study on 61 patients attending AIIMS OPDs who were screened for anemia based on HCS-HLL method and auto-analyzer has been done. The aim was to classify them into
different categories of anemia (mild, moderate and severe) based on both the methods. It was thought that this classification would be enough to decide on the treatment or referral for further investigations. The HCS-HLL kit was found to have very high agreement statistics with the gold standard. Thus this appears to be a promising tool for screening of anemia on a larger scale but warrants further research with a larger sample.

**DISCUSSIONS AND CONCLUSION**

Assessment of hemoglobin is the first step in investigating anemia. When laboratories are not available, anemia is diagnosed based on clinical signs. This entails a lot of inter and intra-observer variations. In rural communities where detection and treatment of anemia is most beneficial, an alternative method, less expensive and reliable is needed.

As per WHO, HCS has a sensitivity and specificity of 95% and 99.5% respectively [25]. However, a study conducted in India [26] and Zanzibar (East Africa) [27] has concluded that HCS should not be used for hemoglobin estimation as the degree of accuracy is not clinically acceptable. In contrast to this another study in Zanzibar [28] and Indonesia [29] recommended HCS in field settings. A recent study in 2014 states that WHO color scale is an inaccurate method to screen anemia during blood donation [30]. It has been proposed that HCS can replace an older copper-sulphate method for screening blood donors for anemia [29,31], as well as Sahli's method in primary health care centers (PHC) or general population [32, 33] and is sufficiently accurate in estimating Hb levels in infants between zero and four months of age [34]. Yet, it is prone to inter-observer variability and has limited utility because it can distinguish only significant levels of anemia as it is not sensitive to detect incremental changes in hemoglobin less than 1g/dl [35].

Sahli's method which is still routinely used in hospital settings has inbuilt disadvantages such as subjective to visual color comparison, need for accurate pipetting, fading of comparator, poor sensitivity and reliability. Comparison of Sahli’s with cyanmethemoglobin has shown that latter is more sensitive and accurate for anemia detection and management in hospitals [36]. However, studies in blood donors have found the sensitivity and specificity of cyanmethemoglobin to be lower than HemoCue [20,21]. Also, there are non-cyanide methods which are much safer than cyanmethemoglobin and with comparable results [17, 37]. Cyanmethemoglobin is the gold standard but its use in surveys in remote areas is limited as it requires accurate dilution of blood sample and electrical power for the spectrophotometer [36].

Automated haematology analyzers have been found to have a higher precision than HemoCue [18]. But this instrument is very expensive and cannot be used at health care facilities in rural areas because of the requirement of a laboratory. On the other hand, NBM-200, a non-invasive method has a lower sensitivity and specificity as well as less correlation with automated analyzer when compared to HemoCue [24]. On the contrary, this non-invasive method was found to be good in terms of precision and screening for blood donors but certain limitations will only be detected with its greater use in different settings and situations [38]. Besides NBM there are other non-invasive methods which have been shown to have less accuracy than HemoCue, but are preferred by blood donors [39-41] except for one study which recommended use of a non-invasive method in ICU [42].

Studies have found the HemoCue method to be more reliable, accurate, rapid, cheap, and easy to handle with no inter-observer variability as manual control is limited to on/off of the battery operated system, compensates for turbidity and has high reproducibility [19-21,43-47]. However, Indian studies have concluded that HemoCue result is an overestimate compared to cyanmethemoglobin and the authors have suggested further studies to validate this method [48,49] whereas in Mexico underestimation of Hb by HemoCue and its poor accuracy has been reported [50]. Sensitivity and specificity of HemoCue varies with the type of blood sample-venous or capillary. Sensitivity and specificity ranges from 42.7-100%; 94.2-100% for venous blood and 56-94.7%; 80.1-100% for capillary blood respectively [20,21,24,51-54]. A study in 1989 showed that HemoCue is a useful instrument for routine screening of iron-deficiency anemia in children; however, for optimal accuracy attention should be given to the method of blood collection [55]. An observational study on critically ill patients concluded that HemoCue measurements are more accurate and reliable when arterial blood is used [56] while capillary hemoglobin values obtained using the HemoCue are higher than venous samples [54,57-60] and should be evaluated with caution when making therapeutic decisions. Other modified versions of HemoCue have also been evaluated which have identical analytical method and can detect Hb at very low concentrations up to 0.02g/dl and have been agreed upon for their use in field [61-63]. A study in Australia has reported that humidity changes the reagents in the HemoCue cuvette which results in underestimation of Hb by 2g/dl. Therefore in tropics, it may result in overestimation of the rate of anemia if proper storage and handling conditions are not maintained [64]. There are certain sources of error which may occur by use of HemoCue such as presence of air bubbles in the light path [19], poor mixing of blood sample if collected in EDTA vacutainers prior to analysis [22] or the microcuvettes [65]. Greater variability due to the above mentioned sources of error has also been demonstrated when same operator takes multiple readings [65,66]. Therefore, it is important to provide proper training to end users. HemoCue is appropriate for surveys that are conducted over large areas and eliminates the need for use of different laboratories allowing screening of anemia in remote areas without adequate lab facilities [21]. Besides screening for anemia it also permits decision making regarding blood donations as it provides on the spot results [20]. HemoCue has also been recommended for monitoring Hb levels in obstetric patients to help guide blood transfusion [67], pediatric surgery [68], operation theatres [69], and liver transplant patients undergoing antiviral therapy [70] and can also aid in measurement of blood loss at caesarean section [71] but should be used with caution in ICU patients [72].

Many methods have been documented in literature but very few of them are suitable for field conditions. So, keeping in mind the attributes of all the methods and thinking from the perspective of developing countries, HemoCue seems to be the method of choice for initial screening of anemia because it is reliable, portable, does not require power supply and easy to use in poor...
resource settings without requiring extensive training of health workers (Table 1). There are recommendations put forward by a review which should be taken into account regarding the use of HemoCue such as addressing economical, clinical and regulatory issues before its implementation, adequate training of end users, and preference for venous and arterial sampling, internal quality control and monitoring of results [73]. For prevention and management of anemia its early diagnosis is necessary; for which a prerequisite is a simple and rapid tool for objective assessment of Hb. However, before making any decision for large scale use of any method one should keep in mind that it costs a considerable amount of money [55,74]. Therefore, methods like HemoCue and HCS-HLL warrant further investigation at large scale and if found to be reliable can be used as tools for screening of anemia in both urban and resource poor settings.

Table 1: Advantages and Disadvantages of different methods for hemoglobin estimation.

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Tallqvist method</td>
<td>Inexpensive, Rapid, Simple, No reagents required, Portable, Electricity not required</td>
<td>Only filter paper supplied in limited quantity can be used. Results influenced by lighting, size and thickness of blood spot, temperature and humidity.</td>
</tr>
<tr>
<td>Copper-sulfate method</td>
<td>Inexpensive, Simple, Rapid, Interpretation objective, Electricity not required</td>
<td>Inaccurate, Provides only ranges of hemoglobin levels, Difficult to conduct in rural areas, Requires fresh solutions, Proper disposal of standard solutions.</td>
</tr>
<tr>
<td>Lovibond comparator</td>
<td>Uses durable device, Useful for routine screening, Electricity not required, Simple, Rapid</td>
<td>Subjective interpretation, Expensive, Requires precise dilution and calibrated pipettes, Needs to be read in natural light, Requires large drop of blood.</td>
</tr>
<tr>
<td>Sahli technique</td>
<td>Simple, Cheap</td>
<td>Inaccurate, Color developed is unstable, Inter-observer variability, Use of manual pipetting-prone to error, No international standard.</td>
</tr>
<tr>
<td>Direct cyanmethemoglobin method</td>
<td>Measurement of cyanmethemoglobin : a stable compound, Standard reference available</td>
<td>Time consuming, Turbidity can deviate the estimate, Cyanide dependent which is toxic.</td>
</tr>
<tr>
<td>Hemoglobin color scale</td>
<td>Simple, Portable, Electricity not required, Cheap</td>
<td>Inter-observer variability.</td>
</tr>
<tr>
<td>HemoCue</td>
<td>Simple, Portable, Rapid-immediate result, Battery operated, Non-toxic, Accurate, Reliable, Easy to use in poor settings where skills and resources are limited</td>
<td>Uses disposable cuvettes which makes it expensive.</td>
</tr>
<tr>
<td>Automated analyzer</td>
<td>Accurate, Reliable</td>
<td>Lab dependent, Transport of samples from the field to lab, Expensive.</td>
</tr>
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REFERENCES


17. Shah VB, Shah BS, Puranik GV. Evaluation of non cyanide methods for


34. van Rhenen PF, de Moort LT. Diagnostic accuracy of the haemoglobin colour scale in neonates and young infants in resource-poor countries. Trop Doct. 2007; 37: 158-161.


52. Muñoz M, Romero A, Gómez JF, Manteca A, Naveira E, Ramírez G.


