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Original Research Article

Assessment of 3-Diferent Haemoglobin Estimation Methods: An Observational Comparative Study

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Abstract:

Aim: The aim of the present study was to assess the different haemoglobin estimation methods.

Methods: This study was conducted on blood samples obtained in 2 ml blood in K3 EDTA vacutainer from various indoor wards and outdoor patient departments in Pathology ,Darbhanga medical College and Hospital, Darbhanga, Bihar, India for one year. 200 adult patients sent for Hb estimation from outpatient and wards of Darbhanga medical College and Hospital, Darbhanga, Bihar, India were included in the study.

Results: Repeatability standard deviations of Sahli's method, Drabkin's method and cell counter respectively were 0.64 g/dl, 0.40 g/dl and 0.16 g/dl. When comparing Sahli's method with Drabkin's method and cell counter, we found p value of <0.0001, suggesting significant difference between two methods whereas Drabkin's method was found to be comparable with cell counter with p value of >0.05. This showed a mean difference of 0.585 and with significant p-value of <0.001. A significant difference was found in the mean values of colorimeter and 5 part ($p<0.001$) despite a significant correlation between these methods.

Conclusion: Sahli's method although cheap and easy, is inaccurate and has subjective bias. So it can be used for screening purpose, but not for diagnosis and follow up of anaemia, Haemoglobin measurement by Drabkin's method is very cost effective and it is as efficient as cell counter. It is especially useful in fund deprived areas and where only haemoglobin value is required. Cell counter although highly accurate and versatile, requires good equipment, quality control, laboratory setup and trained personnel. So it should be preferably used when complete blood count is required.

Keywords: Anaemia, Automated haematology analyser, Haemoglobin estimation, Drabkin's cyanmetheamoglobin method, Sahli's method.

Introduction

Anaemia is a major public health problem in developing countries. One of the reliable indicators for assessing anaemia in population is the determination of haemoglobin concentration. [1] Direct cyanmethaemoglobin method [2] is the most widely used and recommended method by the International Committee for Standardization in Haematology for quantitative estimation of haemoglobin. It involves formation of a stable compound, cyanmethaemoglobin and is relatively a simple and cost-effective method. [3] This direct method involves estimation of haemoglobin in whole blood samples using spectrophotometer and hence requires laboratory facility which limits its applicability in large-scale surveys, as transportation of whole blood in vials from long distances to central laboratories for analysis may not be feasible. Thus, indirect cyanmethaemoglobin (filter paper) method which is based on the same principle but involves spotting of blood on filter paper is often used for the estimation of haemoglobin in population since it is simple and produces reliable results. [4] Due to the minimal invasiveness of sampling and ease of packaging and transportation from long distances, indirect cyanmethaemoglobin has been recommended, especially for those situations where laboratory is located at some distance from blood collection points. [5] Dr Robert Guthrie first used dried blood spot (DBS) specimens to measure phenylalanine in newborns for the identification of inborn errors for the detection of phenylketonuria. [6,7]

Different methods utilized for Hb estimation include acid haematin, photometric cyanmethaemolgobin estimation and automated

estimation with the help of counters. [8] The standard method for measuring hemoglobin (Hb) in human blood is the well-recognized HiCN method as recommended by the World Health Organization (WHO). [9] It is based on photometric detection of cyanmethemoglobin, as an alternative to this technology, HemoCue has developed a photometric method based on the Determination of azide methemoglobin. [10] The cyanmethaemolgobin 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. [11] In India approximately 70% of laboratories still use direct cyanmethaemolgobin method (HiCN) for hemoglobin estimation especially in rural areas. [12] Hemocue method and Cell counter are still costly and require good equipment, quality control, laboratory setup and trained personnel for proper functioning. But in determining the treatment protocol when other RBC indices and complete blood picture are required, automated cell counter is more useful.

The aim of the present study was to assess the different haemoglobin estimation methods.

Materials and Methods

This study was conducted on blood samples obtained in 2 ml blood in K3 EDTA vacutainer from various indoor wards and outdoor patient departments in Pathology Darbhanga medical College and Hospital, Darbhanga, Bihar, India for one year. 200 adult patients sent for Hb estimation from outpatient and wards of Darbhanga medical College and Hospital, Darbhanga, Bihar, India were included in the study.

Samples were taken randomly and their Hb was measured by Sahli's Haemoglobinometer, Cyanmethemoglobin Method &7-part haematology analyzer.

· Hb estimation by Sahli's haemoglobinometer (acid haematin method): Blood is mixed with N/10 HCL, resulting in the conversion of Hb to acid hematin, which was brown in colour. The solution was diluted till its colour matches with the brown coloured glass of the comparator box. The concentration of Hb was read directly.

· Hb estimation by Cyanmethemoglobin Method: Blood is diluted in a solution containing potassium cyanide and alkaline potassium ferricyanide. The latter converts Hb to methaemoglobin which is converted to cyanmethemoglobin (HiCN) by potassium cyanide. The absorbance of the solution is then measured in a spectrophotometer at a wavelength of 540 nm.

If p-value obtained from t-test is >0.05, it means that there is no significant difference between values obtained from both methods and both methods are comparable. While if p value is <0.05, it shows that there is significant difference between results of both methods and they are not comparable.

Results

Table 1: Comparison of Sahli's method, Drabkin's method & Cell counter

	Sahli's method	Drabkin's method	Cell counter
Range of Hb values	12.6-14.2 g/dl	12.7-14 g/dl	13-13.7 g/dl
Mean	13.27 g/dl	13.40 g/dl	13.25 g/dl
Repeatability standard deviation	0.64 g/dl	0.40 g/dl	0.16 g/dl
Method prediction range	12.8-13.7 g/dl	13.05-13.6 g/dl	13.07-13.34 g/dl

Repeatability standard deviations of Sahli's method, Drabkin's method and cell counter respectively were 0.64 g/dl, 0.40 g/dl and 0.16 g/dl. When comparing Sahli's method with Drabkin's method and cell counter, we found p value of <0.0001, suggesting significant difference between two methods whereas Drabkin's method was found to be comparable with cell counter with p value of >0.05.

Table 2: Mean values of Hb obtained using colorimeter and 5 part

Method	Mean	SD	Mean Diff.	t-value	p-value
Colorimeter	13.414	2.755	0.585	3.654	<0.001
5 part	12.780	2.320			

This showed a mean difference of 0.585 and with significant p-value of <0.001. A significant difference was found in the mean values of colorimeter and 5 part (p<0.001) despite a significant correlation between these methods.

Discussion

There are many methods available for hemoglobin (Hb) estimation. In developing countries we are encountered with fund crunch and overcrowded hospitals, so we must design the laboratory method in a way that it should be fast, cost effective and as accurate and reliable as possible. Sahli's method, CuSo4 method and Drabkin's method are very cost effective. Mayang et al, in their study concluded that haemoglobin Concentration should be assessed with the direct cyanmethemoglobin method, the gold standard. [13] The photometer is easy to

transport because it is small and light; it is battery operated and gives consistent results. [14] Photometric determination of haemoglobincyanide (HiCN) is recommended as the reference method. [2]

Repeatability standard deviations of Sahli's method, Drabkin's method and cell counter respectively were 0.64 g/dl, 0.40 g/dl and 0.16 g/dl. When comparing Sahli's method with Drabkin's method and cell counter, we found p value of <0.0001, suggesting significant difference between two methods whereas Drabkin's method was found to be comparable with cell counter with p value of >0.05. This showed a mean difference of 0.585 and with significant p-value of <0.001. A significant difference was found in the mean values of colorimeter and 5 part (p<0.001) despite a significant correlation between these methods. When compared to other studies, Prashant et al 2013[15] found that Sahli's method underestimated the hemoglobin by 1.12gm/dl in venous blood and p value <0.01 between Sahli's method and cyanmethemoglobin method. In a study by P Balasubramanian & A Malathi [16] ,1.13g/dl of difference was found between sahli's method and HiCN method. However a study done by Madhura Wasnik et al using 51 subjects did not find any significant difference between results obtained from Sahli's and HiCN methods (p= 0.954 i.e. >0.05). [17]

Study by Bezarra da silva et al comparing Sahli's method with cell counter did not find any significant difference between the two methods. [18] They found mean difference of 0.2267g/dl. An interesting study done by Dr. MP Brundha and S Priyadharshini, 2019 compared Sahli's two time average and three time average methods with automated cell counter. In this study they found Sahli's three-time average method to be most comparable with autoanalyzer with mean difference of 0.9g/dl. [19]

Conclusion

Sahli's method although cheap and easy, is inaccurate and has subjective bias. So it can be used for screening purpose, but not for diagnosis and follow up of anaemia, Haemoglobin measurement by Drabkin's method is very cost effective and it is as efficient as cell counter. It is especially useful in fund deprived areas and where only haemoglobin value is required. Cell counter although highly accurate and versatile, requires good equipment, quality control, laboratory setup and trained personnel. So it should be preferably used when complete blood count is required.

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