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Original Article

Pathology Section

Haemoglobin Screening Methods in Blood Donors- Where Do We Stand Now?

AASHNA SHIRAZ[1], ASITAVA DEBROY[2]

ABSTRACT

Introduction: Haemoglobin screening is one of the most important screening tests done for blood donors in order to prevent blood collection from an anaemic donor and ensure an optimum quality of blood product for the recipients. Therefore, it is essential, that an accurate and reliable method for haemoglobin determination among the whole blood donors is selected and established.

Aim: To assess the utility and efficacy of the haemoglobin screening methods in blood donors in our institutional setting and to find out the best method taking into account time, cost effectiveness and accuracy of results.

Materials and Methods: This is a single centre-based analytical cross-sectional study of pre-donation screening for whole blood donors in the blood bank of a tertiary care teaching hospital at Durgapur, West Bengal, India. A total of 100 consenting potential donors were screened according to the criteria laid down by Drugs and Cosmetic Act of India, 1940. Blood sample which is routinely collected from the blood donors for screening purpose was tested for haemoglobin estimation using three different screening methods viz., Copper Sulphate method, HemoCue method and Automated Haematology Analyser. The results were analysed using statistical formulae to obtain the specificity, sensitivity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy.

Results: The accuracy of HemoCue (97%) was found to be higher than the Copper Sulphate method (92%). Comparing the cost per test, Copper Sulphate method was the most affordable with 5 INR/test, HemoCue and Automated Haematology Cell Analyser at 25 INR/test each. Taking into account the time taken by the methods, we found that HemoCue took the least time for screening a blood sample, followed by the Copper Sulphate method.

Conclusion: Taking into account the efficacy, utility, time taken and cost effectiveness, HemoCue was found to be an appropriate screening method for pre-donation estimation of haemoglobin content in majority of blood donation setups. However, in economically poor areas, Copper Sulphate method can be used but HemoCue method should be employed for those who fail the former method, so as to avoid unnecessary deferrals.

Keywords: Accurate, Best method, Cost-effective, Haemoglobin estimation

INTRODUCTION

To provide safe and quality blood products for the patients is one of the primary goals of a blood transfusion service and to ensure this, we have to prevent blood collection from an anaemic donor. Therefore, pre-donation haemoglobin testing for blood donors is very important in order to safeguard the health of the transfusion recipients [1].

It is, therefore, essential that there should be an accurate and reliable method for haemoglobin determination among the whole blood donors. According to the Indian Drugs and Cosmetics Act, 1940, for blood donation, the minimum acceptable haemoglobin is 12.5 g/dl or a haematocrit of 38% for both males and females [2].

Inspite of having various methods for haemoglobin estimation, no single technique has emerged as the most appropriate and ideal for a blood donation set-up. A highly accurate method in a blood donor setting is more likely to be expensive. In a developing country like India, it is not possible to use such method for screening of such a large number of donors' blood sample. On the other hand, a less accurate and cheaper method may give false results which may lead to either donation of blood by an anaemic subject or loss of eligible donors. Therefore, there is requirement of adopting a method that delivers accurate laboratory results along with being cost effective and time saving [3].

Copper sulphate specific gravity method has been traditionally used and is still being used for donor screening at many blood centers in India because of its easy availability and cost-effectiveness. But it does not provide an acceptable degree of accuracy [4,5]. Many studies have shown that the subjects who are deferred because of failing the test may not actually be anaemic [6]. It is therefore, important to determine anaemia amongst them using the standard diagnostic method so that there is no loss of any potential donors.

Keeping into account all these factors, this study was designed to assess the utility and efficacy of the haemoglobin screening methods in blood donors viz., copper sulphate method, HemoCue method and Standard Haematology Analyser method (Sysmex- KX 21) in our institutional setting and to find out the best method taking into account time, cost-effectiveness and accuracy of results.

MATERIALS AND METHODS

This is a single centre-based analytical cross-sectional study of pre-donation screening for whole blood donors in the blood bank of a tertiary care teaching hospital at Durgapur, West Bengal, India, over a period of two months, i.e., from 1st June 2016 to 31st July 2016 as a part of an approved Short Term Studentship Project by Indian Council of Medical Research. Ethical clearance for this study was given by the Institutional Ethics Committee (Ref. No: IQMC/IEC/APRW/16/002).

A total 100 consenting potential donors were screened (continuous sampling) according to the criteria laid down by Drugs and Cosmetic Act of India, 1940 for the purpose of blood donation. Blood sample which was routinely collected from the blood donors for screening purpose was used for the study. The blood was tested for haemoglobin estimation using three different screening methods as mentioned above.

Blood donor deferrals were made according to standard guidelines [7]. Reasons for deferral were donors who were less than 18 years or more than 65 years of age, weight less than 45 kg, had undergone any major surgery in the last one year or minor surgery in the last

six months, taking any specific medications like antibiotics, aspirin, antithyroid drugs etc., had history of unprotected sex with multiple sexual partners and many more.

We have taken the readings of Automated Haematology Cell Analyser (Sysmex- KX 21) as reference values and compared the readings of copper sulphate method and Hemocue method with its values. Sysmex KX-21 has proved to be an ideal haematology analyser for any clinic satellite laboratory or research testing. It provides a Complete Blood Count (CBC) with 17 reportable parameters and 3-part WBC Differential. The use of automatic floating discriminators provides a high level of accuracy to the system.

The Sysmex KX -21 uses sodium lauryl sulphate method for haemoglobin analysis, which is a non- cyanide method with very short reaction times. Haemoglobin is determined in a separate channel, minimizing interference from high leukocyte concentrations.

Principle of the Tests

Copper Sulphate Method: It is based on specific gravity of blood. A blood droplet is allowed to fall into copper sulphate solution of a specific gravity 1.053 and the movement of droplet is observed for atleast 15 seconds [8,9].

HemoCue Method: This is a non-dilution system where Sodium deoxycholate haemolyses the erythrocytes and haemoglobin is released. Haemoglobin is converted to methaemoglobin by sodium nitrite which, together with sodium azide, gives azide-methaemoglobin. The absorbance is measured at two wavelengths (570 nm and 880 nm) [10-12].

Automated Haematology Cell Analyser: It uses a non-cyanide haemoglobin analysis method which measures both oxymethaemoglobin and methaemoglobin [13].

Laboratory Procedure: The donor's finger was pricked only once and two drops of blood was collected for haemoglobin estimation by two different methods (copper sulphate and hemocue) as stated below. Blood for haemoglobin estimation by automated haematology analyser is collected in EDTA vial during the process of blood collection only.

For Copper Sulphate method: One drop of blood (0.05 ml approx) is produced from the site of an alcohol swabbed finger of the blood donor. The working copper sulphate solution (of specific gravity 1.053) is taken in a clear beaker, having depth of at least 3 inch. A drop of blood is then allowed to fall into 30mL solution from a height of 1 cm. If the haemoglobin content is more than 12.5 g/dL, the droplet of blood sinks and if it is less than 12.5 g/dL, it floats. However, the result is declared only after 15 seconds of careful observation.

For Hemocue Method: A small drop of blood (0.01 ml approx) is produced from the site of an alcohol swabbed finger of the blood donor. The drop of blood is collected in the self-filling disposable cuvette supplied by the manufacturer and fed into the machine. The results are displayed within 60 seconds. The haemoglobin measuring range is 0-25.6 g/dL.

For Automated Haematology Cell Analyser: A 2mL of blood along with dipotassium EDTA, collected during phlebotomy, is used. All specimen tubes are mixed thoroughly on a mechanical mixer for at least 2 minutes and then run on the analyser. The results are displayed within 60 seconds.

STATISTICAL ANALYSIS

The objective was to obtain the specificity, sensitivity, PPV, NPV and accuracy of the two methods viz., Copper sulphate method and HemoCue method in comparison with the reference standard of Automated Haematology Analyser. Therefore, the data were analysed using SPSS 20.0

RESULTS

Results were recorded in separate laboratory registers and results of the sensitivity, specificity, PPV, NPV, percentage of false negatives, percentage of false positives, accuracy for copper sulphate method and HemoCue were calculated manually. Results of copper sulphate method were interpreted as pass or fail at haemoglobin cut-off of ≥12.5 g/dL. Data was represented as frequency tables and graphs for comparison, taking Automated Haematology Cell Analyser as gold standard.

We did the cost analysis and analysis of the maximum time taken by the three screening methods, i.e., Automated Haematology Cell Analyser, HemoCue and Copper Sulphate method for determination of feasibility for their implementation in a blood donor setup.

The gender distribution of 100 donor population predominantly consisted of males with only 9% female representation. Donor's age ranged from 18 to 57 years, majority of them belonging to 28-37 age group [Table/Fig-1].

Age distribution of donors (in yrs)	Total No of donors	Gender distribution		No of deferrals (n=16)	
		Male	Female	Male	Female
18-27	36	34	2	2	0
28-37	37	33	4	3	1
38-47	23	20	3	6	2
48-57	4	4	0	2	0
Total--->	100	91	9	13	3

[Table/Fig-1]: Age and gender distribution of blood donors along with the number of deferrals.

Among these 100 donors, 16 were deferred due to low Haemoglobin content as recorded by the Automated Haematology Cell Analyser. Out of these deferrals, 13 were males (81.25%) and rest was females [Table/Fig-1]. The haemoglobin level of the true deferrals was found to be between 10.9-12.4g/dL.

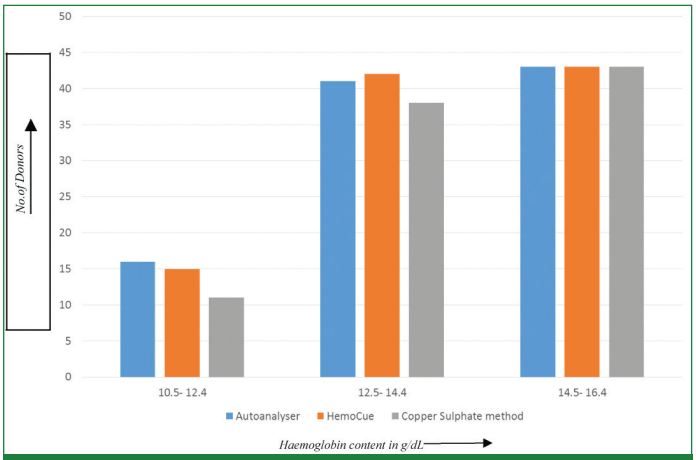
The overall mean haemoglobin content was 13.8 g/dL for Automated Haematology Cell Analyser and 13.9 g/dL for HemoCue. Mean value of HemoCue (mean±SD = 13.9±1.5 g/dL) was higher by 0.12 compared to Automated Haematology Cell Analyser.

Among the 16 true deferrals, the HemoCue detected 15 of them whereas Copper Sulphate method detected only 11 of them as true deferrals. Also, Copper Sulphate screening test inappropriately passed 5 donors (31.25%), who had haemoglobin values between 10.9-12.4 g/dL when tested by Automated Haematology Cell Analyser, while 3 donors (3.57%) were falsely deferred by this method. Different methods used in the present study are compared in [Table/Fig-2,3].

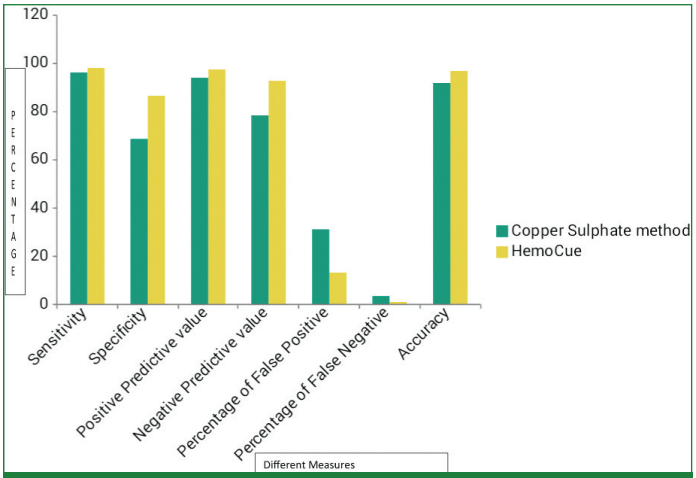
Interval (Hb in g/dL)	Autoanalyser (gold standard)	Hemo Cue	Copper sulphate method	
			sinking droplet (Pass)	floating droplet (Fail)
10.5-12.4 (True deferral)	16	15	05	11
12.5-14.4	41	42	38	03
14.5-16.4	43	43	43	00
Total	100	100	100	

[Table/Fig-2]: Comparative study of haemoglobin values of donors obtained by three different methods.

The sensitivity of HemoCue and Copper Sulphate method was found to be 98.28% and 96.42% and their specificity was 86.66% and 68.75% respectively. The PPV of HemoCue and Copper Sulphate methods were 97.67% and 94.18% respectively and their NPV were 92.85% and 78.57% respectively. The percentage of false positive and false negative values of HemoCue was lower (13.33% and 1.17% respectively) as compared to that of Copper Sulphate method (31.25% and 3.57% respectively). Also, the accuracy of HemoCue (97%) is found to be higher than the Copper Sulphate method (92%) [Table/Fig-4].



[Table/Fig-3]: Showing distribution of haemoglobin concentration among the blood donors as obtained by three different methods.



[Table/Fig-4]: Comparison of different statistical measures between Copper Sulphate method and HemoCue.

DISCUSSION

For an effective blood transfusion service, it is essential to have an appropriate haemoglobin screening method so as to accept maximum suitable donors and to prevent any unnecessary deferrals. Ideally such a method should be time saving, cost effective and must be validated against Automated Cell Counters [3].

In developing nations, the semi-quantitative gravimetric copper sulphate method which is very easy and inexpensive is used in many blood centers, but does not provide an acceptable degree of accuracy.

In our study, Copper Sulphate method was found to have 96.42% sensitivity and 68.75% specificity which are quite similar to the observations made by Gupta S et al., [1]. Also, we found that this screening method inappropriately allowed 31.25% of donors who were actually anaemic. This observation is similar to that made by Sawant RB et al., (32.8%) [14]. Similarly, Boulton FE et al., observed such inappropriate passes being within 1.0 g/dl of the threshold for their gender, by this method [15]. Studies conducted by Tondon R et al., and James V et al., also showed significant number of such donors [3,16]. Some cases in which plasma protein concentration is greatly raised, anaemic donors may be accepted as normal by this method. False positive results may also be due to high white cell count [1].

Many studies have suggested that this method is also likely to give inappropriate failures and a significant number of such failed donors could be recovered with alternative method of screening [17]. In our study, we had 7.32% of such cases of inappropriate failure by copper sulphate screening method.

The HemoCue is a very handy machine which does not require any pre-treatment of the blood sample and background turbidity of the samples is corrected due to the measurement of two-wave lengths [11]. Moreover, this method is time-saving (takes only 60 seconds), very easy to operate and thus, gives a shorter turn-around time. It also requires very less amount of capillary or venous blood (only 10 µL), cost effective and a more accurate method [18].

Comparing the cost per test, Copper Sulphate method was the most affordable with 5 INR/test, HemoCue and Automated Haematology Cell Analyser at 25 INR/test each. 500 g of Copper Sulphate method costs only 255 INR and 159.63 g can be used approximately for 700-800 tests. On the other hand, the HemoCue instrument and each disposable microcuvette itself costs 28,000 INR and 30 INR respectively. Also, an Automated Haematology Cell Analyser costs around 3,99,000 INR.

Taking into account the time taken by the methods, we found that HemoCue took the least time for screening a blood sample, followed by the Copper Sulphate method. The detailed study of the maximum time taken by the methods is given in [Table/Fig-5].

Procedures	Autoanalyser	HemoCue	Copper Sulphate
Pre-testing	5	1	1
Testing	1	1	2
Post-testing	2	0	0
Total	8	2	3

[Table/Fig-5]: Maximum time taken (in minutes) by the three different methods for screening one blood sample.

In our study the sensitivity of HemoCue was found to be 98.28% which is similar to the results found by Tondon R et al., Sawant RB et al., and Boulton FE et al., [3,14,15]. The values obtained by HemoCue were found to be higher than actual haemoglobin by 1.5g/dL, which is similar to the results obtained by Deb R et al., [19]. However, HemoCue gave higher mean of haemoglobin values when it was compared with the reference value. In our study, 1 such donor (constituting 1.19%) was re-accepted by this method who was deferred by Automated Haematology Cell Analyser. Thus, this method might actually include anaemic donors which might ultimately affect the product quality and be harmful to the donor [11].

Copper Sulphate method is the method of choice in many centres across the country for primary haemoglobin screening of potential blood donors since many years. This is because of the fact that it is relatively inexpensive. However, intense training and constant monitoring of the staff, performing this method, is necessary. Also, strict quality control and validation should be done. Also, we found that it does not give quantitative result of haemoglobin and has a chance of false acceptance and deferral [1].

On the other hand, HemoCue haemoglobin photometer is a portable, battery operated device, which can be used as a point-of care device for haemoglobin estimation for more or less accurate results. Also, it is user friendly, needs minimum training, gives result in no time [20].

Thus, we can say, that it is very much convenient to use HemoCue as haemoglobin screening method prior to donation of blood in tertiary set-ups, relatively remote areas and in mobile donations as it time saving and cost effective. However, Copper Sulphate method stands the test of time and it can be continued as the primary method in areas of economic crisis as using HemoCue in such places can prove to be costly. But those who fail the copper sulphate procedure of haemoglobin testing should not be just sent as deferred donors but rather be assessed by HemoCue to save inappropriate deferrals. Also, rigorous training of the staff, for using Copper Sulphate method, is required to get maximum possible efficacy.

LIMITATION

The study period was only of two months as it was a part of ICMR-STS project. The same study done over a longer period of time will give a stronger statistically significant data.

CONCLUSION

Approximately 8 million blood donations are collected annually in India. Hence, even a small percentage of false acceptance or false deferral by the haemoglobin screening represents a significantly large number of individuals. So, it is very important to have an accurate screening test for determination of haemoglobin content prior to donation.

Taking into account the efficacy, utility, time taken and cost effectiveness, it can be inferred that HemoCue is an appropriate screening method for pre-donation estimation of haemoglobin content for majority of blood donation set-ups. However, in economically poor areas, Copper Sulphate method may be used but HemoCue method should be kept as a back-up method for those who fail the former, so as to avoid unnecessary deferrals.

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PARTICULARS OF CONTRIBUTORS:

1. Intern, MBBS, IQ City Medical College, Durgapur, West Bengal, India.
2. Associate Professor and Dean Academics, Department of Pathology, IQ City Medical College, Durgapur, West Bengal, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Asitava Debroy,
IQ CITY Medical College, Durgapur, West Bengal, India.
E-mail: asitavadr@gmail.com

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