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RESEARCH ARTICLE

COMPARATIVE STUDY OF HAEMOGLOBIN ESTIMATION OF BLOOD DONORS BY SPECIFIC GRAVITY (CuSO4), HEMOCUE & AUTOMATED CELL COUNTER METHODS

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ABSTRACT

The Hb estimation of blood donor is the only laboratory test performed prior to blood donation and is of paramount importance. The main objective of our study was to compare the efficacy of the three common haemoglobin estimation methods, namely, $CuSO_4$ method, HemoCue photometer & Automated Cell Counter in reporting the haemoglobin levels of blood donors. This prospective study was conducted on 500 random voluntary non-remunerated altruistic blood donors over a period of 3 months. Copper sulphate results were interpreted as pass or fail, digital readings were obtained for the other two methods. The sensitivity, specificity, positive and negative predictive values of each method was calculated. Hb screening by $CuSO_4$ is an inexpensive and convenient method to be used as for primary screening, supplemented with HemoCue for donors rejected by CuSO4.

INTRODUCTION

The haemoglobin estimation of blood donor is the only laboratory test performed prior to blood donation and is of paramount importance. Pre-donation haemoglobin screening is used both to safeguard the health of potential donors and to ensure an adequate quality of blood products for recipients. According to the Indian Drugs and Cosmetics Act, 1940 for blood donation, the minimum acceptable haemoglobin (Hb) is 12.5 g/dl or haematocrit (Hct) of 38% for both males and females [1].

The primary purpose of Hb screening is donor protection: preventing an anemic individual from exacerbating their condition with ill effects. The second purpose is to ensure the patient receives a minimum infused Hb dose per Red Blood Cell transfusion[2].

Various methods of haemoglobin estimation have evolved over the period of years, from the simplest Hb test, the Tallqvist method [3], in which the colour of blood in blotting paper was compared with a colour scale to measurement by photometer and the new proposed Hb estimation methods without even giving finger prick (occlusion spectroscopy), each with its own advantages and limitations.

Despite the availability of various methods for measuring donor haemoglobin, no single technique has emerged as the most suitable for haemoglobin testing in a blood donation setting. Validity of these methods has to be evaluated before use and methods with sufficient sensitivity and specificity should be used in order not to expose blood donors and recipients to risk or to lose potential donors.

Numerous studies have been done to evaluate the diagnostic value of these rapid methods for determining low haemoglobin levels and anaemia [4-9]. The goal of these studies was to select highly sensitive and accurate methods with very low false-deferral and false-pass rates.

The main objective of our study is to compare the efficacy of the three common haemoglobin estimation methods, namely, Specific Gravity Method using Copper Sulphate, HemoCue haemoglobin photometer and Automated Cell Counter (haematologyanalyzer) in reporting the actual haemoglobin levels of blood donors.

MATERIALS AND METHODS

This prospective study was conducted on 500 random voluntary non-remunerated altruistic blood donors over a

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period of 3 months. All participants were fully informed of the aim of the study and their consent was obtained. Ethical clearance was obtained from the institute board.

Capillary blood samples were collected by deep finger prick on the index or middle finger of left hand using a dry sterile lancet (UniletExcelite II, England) after disinfecting with ethanol and massaging the finger to facilitate blood flow. The first drop was wiped away and the second drop was used for testing by copper sulphate method and HemoCue method (HemoCue® AB, Ängelholm, Sweden). Two millilitres of venous blood samples were collected into EDTA vacutainer tubes and were analysed on the automated cell counter as soon as possible.

Blood sampling and analysis of Hb was performed only by doctors and technicians who were trained for the instruments on a few pilot samples using the three methods before commencing the study.

The $CuSO_4$ method uses the principle that a drop of whole blood dropped into a solution of $CuSO_4$, which has a given specific gravity, will maintain its own density for approximately 15 seconds. The test solution should have a specific gravity of 1.053.[10] Errors in techniques in using the CuSO4 method, for example, incorporation of air bubbles or the use of an inadequate height for dropping the blood, tend to result in underestimating the Hb concentration so that donors may be deferred unnecessarily. Low or high proteins in the donor may also lead to false results[11].

The HemoCue® B-Hemoglobin system (HemoCue® AB, Ängelholm, Sweden) consists of disposable micro-cuvettes containing reagent in a dry form and a single purpose designed photometer. The micro-cuvettes were stored in a dry place at room temperature. Once opened, they were tightly closed and stored at the same conditions to maintain their integrity and shelf life. The reaction in the microcuvette is a modified azide-methemoglobin reaction. Sodium deoxycholate haemolyses erythrocytes and haemoglobin is released. Sodium nitrite converts haemoglobin to methemoglobin which, together with sodium azide, gives azidemethemoglobin. The absorbance is measured at two wavelengths (570 nm and 880 nm) in order to compensate for turbidity in the sample. The test was performed as stated by the manufacturer [12].

The automated blood cell counter (ERMA PCE 210, Tokyo) is intended for in vitro diagnostic use in clinical laboratories. It is a compact, fully automated hematologyanalyzer with simultaneous analysis of 18 parameters. It was taken as reference method against which all the other methods were tested.

Quality Control

The working $CuSO_4$ solution was prepared (specific gravity 1.053) and was standardized before use according to the standard operating procedure (SOP).

The HemoCue (HemoCue® AB, Ängelholm, Sweden) instrument is factory calibrated and its quality control is done quarterly.

The automated blood cell counter (ERMA PCE 210, Tokyo) is calibrated annually and its quality control was done before use with the stabilized control reagents provided.

Statistics

Results of copper sulphate were interpreted as pass or fail at Hb cut-off of $\geq$ 12.5 g/dl. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) of each method was calculated.

RESULTS

Donor's age ranged from 19 to 58 years. The gender distribution among 500 donor population predominantly consisted of males (69.8%) with comparatively less female population (30.2%). The deferral rate was higher among female donors (72.0%) as compared to male donors (28.0%)[Table 1].

Table 1 Demographic Characteristics of Donors (by reference method)

Gender	Pass	Fail	Total number
Male	335(74.44%)	14(28%)	349(69.8%)
Female	115(25.55%)	36(72%)	151(30.2%)
Total number	450 (90%)	50(10%)	500

Among 500 blood donors 50 were deferred (10%), the deferral rate was more in first time donors (56%) as compared to repeat donors (44%)[Table 2].

Table 2 Frequency of donations among the donors accepted/deferred (by reference method)

Donation Status	Pass	Fail	Total number
First time	104(23.1%)	28(56%)	132 (26.4%)
Repeat donors	346(76.8%)	22(44%)	368 (73.6%)
Total number	450(90%)	50(10%)	500

The minimum value of Hb among blood donors was 9.5g/dl while the maximum being around 17g/dl.Haemoglobin values with all the methods were analysed and comparison of the different methods used in the present study against the reference hematologyanalyzer is summarized [Table 3].

Table 3 True deferral data from the reference cell counter

Hb values	True deferral (by analyzer)	$CuSO_4$		HemoCue	
		Pass	Fail	Pass	Fail
9.5 – 10.9	9	0	9	0	9
11.0 – 12.4	41	19	22	8	33
12.5 – 13.9	244	239	5	243	1
14.0 – 15.9	185	183	2	185	0
16.0 – 17.0	21	21	0	21	0

HemoCue was found to be more efficient with a Sensitivity of 99.77%, Specificity of 84%, PPV - 98.24% and NPV - 97.6%. CuSO4 was found to be less specific with, Specificity -55.1%, Sensitivity - 96.81%, PPV - 91.86% and NPV - 81.42%.

The $CuSO_4$ screening test inappropriately passed 19/500(3.8%) donors, out of these 17 donors had Hb values between 11.0-12.4 g/dl when tested by Cell Counter, while 07/500 (1.4%) donors were falsely deferred by CuSO4 method. Different methods used in the present study are compared in [Table 4].

Table 4 Performance characteristics of three methods for Hb estimation of blood donors

Result	$CuSO_4$	HemoCue
True positive	443	449
True negative	31	42
False positive	19	8
False negative	7	1
Sensitivity (%)	98.40%	99.70%
Specificity (%)	62%	84%
Likelihood ratio	257.89	618.75
Positive predictive value	95.80%	98.24%
Negative predictive value	81%	97.60%

DISCUSSION

The semi-quantitative gravimetric copper sulphate method, being very easy and inexpensive is the traditional method being used for donor screening at many blood centers. It has been the method of choice in every country for primary Hb screening of potential blood donors for many years. It is inexpensive, fast, and does not require venous sample. However, rigorous training and constant observation of staff is necessary. It needs to undergo strict quality control and validation before it is used to screen the donors. It doesn't give quantitative result of Hb and always has a chance of false acceptance and deferral. Early reports suggested that this method tended to give inappropriate failures, and a significant number of such failed donors could be recovered with alternative method of screening[2]. On the other hand, rare cases in which plasma protein concentration is greatly raised, anaemic donors may be accepted as normal by copper sulphate method, each extra g/dL of plasma protein being equivalent to 0.7 g/dL Hb. Falsely high positive results in $CuSO_4$ sulphate method is also due to high white cell count.

In our study $CuSO_4$ method inappropriately passed 3.8% of donors of which a majority were within 1.0g/dl of threshold against the reference values, which is quite similar to observations made by Rashmi *et al*[16] and James *et al*[7].Similarly Boulton *et al*[17] observed more inappropriate passes by $CuSO_4$ method with inappropriate passes being within 1.0 g/dl of the threshold for their gender.

The HemoCue haemoglobin photometer is a portable, battery-operated photometric device[5], being widely used as a point-of-care device for haemoglobin estimation in mobile blood donations and critical care areas in health facilities. HemoCue also has an additional advantage over other photometric methods in that it incorporates a turbidity control, due to which more accurate results on lipaemic samples is obtained[13-15]. It is a good method of performing haemoglobin testing in blood donors, but its drawback is that it is very expensive. It is important to carefully train the staff in the filling of the cuvettes, because air bubbles and fingerprints or blood on the cuvette face can give erroneous readings.

In our study the sensitivity of HemoCue was found to be 99.77% which is similar to the results found by Sawant *et al* [8], Boulton *et al*[17], Rashmi *et al*[16], Chambers *et al* [18].

The Hb values obtained by HemoCue haemoglobinometer were found to be higher than actual Hb by 1.2g/dl, which is similar to the results obtained by Deb R *et al* [19]. HemoCue is simple to use, needs minimum training, and gives an immediate result. It is useful in clinical and epidemiological settings where finger puncture allows capillary blood sampling as an easy technique which is less resource-intensive than vein puncture, and is more acceptable to patients and the community.

CONCLUSION

The method used for Hb screening of blood donors should be reliable and affording.

Hb screening by $CuSO_4$ still stands the test of time and it can be used as the primary screening method. Using HemoCue as the initial screening method could prove costly for some blood centers. The Hb level of donors rejected by $CuSO_4$ may be reassessed by HemoCue, to decide whether or not the donor needs to be actually deferred. This finding could be of value to blood centers with limited resources especially for camp donations where mass donor haemoglobin screening is carried out.

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