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ORIGINAL ARTICLE

Evaluation of the Performance of Copper Sulphate and Hemocue Methods for Haemoglobin Estimation Among Blood Donors in Dar Es Salaam, Tanzania

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ABSTRACT

Background: The National Blood Transfusion Service (NBTS) in Tanzania uses the Copper Sulphate ($CuSO_4$) gravimetric method to estimate hemoglobin (Hb) in blood donors. However, this and other point-of-care methods, including HemoCue, may provide false results. Therefore, this study aimed to evaluate the performance of $CuSO_4$ and HemoCue methods for Hb estimation compared with automated haematology analyzer (AHA).

Methods: The cross-sectional study was conducted among (N=204) blood donors in Dar es Salaam. Capillary blood samples were obtained for Hb estimation by $CuSO_4$ and HemoCue methods, 3 mls of venous blood were also collected for Hb quantification by AHA (gold standard), HemoCue and $CuSO_4$ gravimetric method. Data were analyzed by Epi info 7.2.2.6, statistical significance was defined at a P value of <0.05, and kappa agreement was calculated.

Results: The median age of the study participants was 30 years (IQR: 20-39). The proportion of false eligible donors was 19.6%, and false deferral donors were 2.9% by the $CuSO_4$ gravimetric method. The specificity, sensitivity, positive and negative predictive values, and Kappa agreement for $CuSO_4$ were 28.6%, 95.9%, 78.0%, 72.7%, and 0.1, respectively. In contrast, the specificity, sensitivity, positive and negative predictive values, and Kappa agreement for HemoCue were 62.5%, 98.6%, 87.4%, 94.6%, and 0.63, respectively.

Conclusion: Our study revealed that the performance of the $CuSO_4$ gravimetric method in Tanzania is relatively poor, with a high proportion of false eligible donors than the HemoCue method. These findings warrant further studies to evaluate the quality control measures for $CuSO_4$ gravimetric method and explore alternative point-of-care methods for Hb estimation among blood donors in similar resource limited-settings.

BACKGROUND

Haemoglobin (Hb) screening is mandatory to safeguard donors' health and ensure adequate blood supply to recipients. Hb screening, when performed appropriately, correctly identifies eligible donors who qualify to donate blood based on the set criteria.[1-3] Eligible blood donors should have a Hb concentration of more than 12.5 g/dL because one loses 0.7 g/dL to 1.5 g/dL of Hb following donation.[4] While the automated haematology analyzer (AHA) is the gold-standard test for quantifying Hb among blood donors, resource-limited settings use various point-of-care tests such as the Copper sulphate ($CuSO_4$) gravimetric and HemoCue methods to estimate Hb. However, the point of care methods may inappropriately designate an eligible donor as a deferral (not eligible) donor. Therefore, the methods used to estimate Hb should accurately detect the threshold of 12.5 g/dL.

In Tanzania and other resource-limited setting, many blood donation centers use the $CuSO_4$ gravimetric method to estimate Hb in blood donors. This method is known to be easy to perform, quick and cost effective. However, the method is known to be affected by high serum protein, high leucocyte count, and high ambient temperature, while waste disposal of the solution used is a considred a biohazard, and in some countries, it is regarded an environmental toxin; and most importantly the method cannot quantify the exact amount of Hb.[4] Moreover, there is a lack of a generally accepted quality control for the method and the fact that it cannot quantify the exact amount of Hb, therefore, it is not feasible to detect an abnormally low or high Hb level.[5-7] Hence, it may potentially provide false results, leading to donation-induced iron deficiency anemia[8-11] and the loss of blood donors.[12-14]

The proposed mechanism for this shortcoming is that

$CuSO_4 5H_2O$, when poorly reconstituted, can precipitate Hb, other proteins, and leukocytes.[10] Due to these shortcomings blood transfusion centers in other developed countries have shifted towards other point-of-care methods of Hb estimation such as HemoCue.[15-17] Despite these shortcomings, the CuSO4 gravimetric method is the recommended method for pre-donation Hb screening in resource-limited settings.[18]

There is a paucity of information regarding the performance of the $CuSO_4$ gravimetric method in Tanzania compared with other available point-of-care Hb estimation methods, such as the HemoCue, in estimating the Hb threshold among blood donors. Therefore, the present study evaluated the performance of $CuSO_4$ and HemoCue methods for estimating Hb among blood donors in Dar es Salaam, Tanzania.

METHODS

The study design and setting

This was a cross-sectional study conducted within a period of 3 months from January to March 2019. The study was conducted in three blood donation centers, namely Muhimbili National Hospital (MNH), Eastern Zone Blood Transfusion Centre (EZBTC), and Temeke blood transfusion satellite site in Dar es Salaam, Tanzania. According to the Tanzania National Blood Transfusion Service (NBTS) annual report, the selected sites contributed 90.2% of blood donated in Dar es Salaam region from January to December 2017.

Study Population, Sample Size, and Sampling Procedure

The study population was clients above 18 years of age who visited the centers for routine blood donation. The sample size was estimated using the formula for testing the sensitivity (or specificity) adopted from Hajian-Tilaki *et al.* 2014.[19] The largest sample size was 183 and was selected as the minimum number of participants required in this study, corresponding to $CuSO_4$ gravimetric method sensitivity of 98.4 %.[20] In addition, the estimate included a 10% non-response rate for a final target sample size of 204 participants.

The number of study participants for each blood donation center was selected according to probability proportional to size (PPS) sampling, whereby at MNH (n=80), EZBTC (n=64), and Temeke (n=60). We used systematic sampling to select the interval between blood donors. The time of data collection was 40 days with minimum sample size of 10 participants expected per site per day. Hence we used a formula k=population (400)/sample size(204)= 1.96 ≈ 2. Therefore, every second participant was sampled from the blood donors during the working hours until the estimated sample size was obtained.

Sample Collection Procedure

Capillary blood samples were obtained from prospective blood donors by lancing a fingertip on the index or middle finger of left hand using a dry sterile lancet after disinfecting with ethanol and massaging the finger to facilitate blood flow of a seated prospective blood donor. The first drop of blood was wiped off, while the second and third drops were collected into a capillary tube for testing using $CuSO_4$ gravimetric method and microcuvette for the HemoCue method in alternating order.[21, 22] Ethylene Diamine Tetra- Acetic acid (EDTA) anticoagulant test tubes were labelled with two identifications that of the blood donation centre and the study identification. Then three milliliters (3 ml) of venous blood were collected aseptically from each study participant into the EDTA tubes and transported to Muhimbili National Hospital at a temperature of 2^0 C to 8^0 C in a cool box with ice packs by a trained laboratory research assistant. All the samples were transported everyday within 2 hours of being collected along with the study sample laboratory request form, sample manifest and sample tracking form. The venous samples were analyzed for Hb by using an automated haematology analyzer (Abbott Cell-Dyn 3700, MN, USA), HemoCue and CuSO4 gravimetric methods.

Sample Processing

Collected capillary blood samples were directly measured onsite for Hb by using $CuSO_4$ gravimetric and HemoCue methods.[21, 22] The 3 mL venous samples collected into each EDTA tube, was gently mixed 3-5 times then 0.5 mL was aspirated for Hb estimation by HemoCue and CuSO4 gravimetric methods and the remaining volume was used for Hb testing by using an automated haematology analyzer.

Quality control (QC)

Copper sulphate pentahydrate ($CuSO_4 5H_2O$) preparation was done following the World Health Organization (WHO) Standard Operating Procedures (SOPs) to ensure quality of the $CuSO_4$ solution.[23] Briefly 170 gram of crystalline $CuSO_4$ powder was dissolved in 1000 mL distilled water to make a stock solution of $CuSO_4 5H_2O$. Then mixed well to ensure that the copper sulphate has dissolved. Then 51 ml stock solution was added into 49 mL distilled water to make a working solution. The specific gravity (1.053) was checked using a hydrometer, if 1.053 gravity was not obtained, it was adjusted by either using stock solution or distilled water.[23]

The calibration of the HemoCue was verified by a control cuvette each day before the first measurement as recommended by the manufacturer. In short, QC was performed daily as recommended by the manufacturer and the liquid QC testing was conducted prior to clients sample testing. This QC test ensures the accuracy of the HemoCue analyzer. The liquid QC comes in two levels: R&D GLU/HGB Control Level 1 (low) and R&D GLU/HGB Control Level 2 (high). In addition, the HemoCue analyzer has an Internal Electronic Quality Control (EQC) that is performed automatically each time the device is turned on. This test verifies the performance of the optronic unit of the analyzer. This test is performed eight hourly when the analyzer remains powered on. An automated Full Blood Picture (FBP) machine (Cell dyne 3700 analyzer) calibration and control were performed each day as recommended by the manufacturer. The QC for Abbott Cell-Dyn 3700, has an in-built internal quality control system and is conducted before running any patient samples after the verification that the background counts displayed are within the acceptable ranges as per manufacturer's instruction.

Data Analysis

Data were entered, cleaned, and stored in Microsoft (MS) Excel version 2019, and control of data quality was

conducted through the review of data collection tools. Then the data were exported into Epi Info version 7.2.2.6 for statistical analysis. The data set copy backup was made for any occasion that may need backup during data analysis.

The categorical variables were presented in frequency and proportions, whereas normally distributed continuous variables were presented as means with Standard deviations (SD), and those not normally distributed were presented as medians with interquartile ranges (IQR). The performance of both $CuSO_4.5H_2O$ and HemoCue was estimated by calculating the sensitivity, specificity, positive and negative predictive values, and kappa agreement with results from automated haematology analyzer as reference or gold-standard method.[20, 24, 25]

We defined sensitivity as the percentage of donors with Hb values below the cut-off of 12.5 g/dl (failed) identified by the test out of all donors with venous Hb values below the cut-off by the gold-standard test.[4] We calculated specificity as the percentage of donors with Hb value above the cut-off of 12.5 g/dl (those passed) identified by the test out of all donors with venous blood Hb above the cut-off value by gold-standard test.[4] Positive Predictive Value (PPV) was defined as the probability for a donor to have a Hb value below cut-off (failed/deferral) by both the test as well as the reference method.[4] Negative Predictive Value (NPV) was defined as the probability of a donor to have Hb value at or above the cut-off (passed/eligible) by both the test as well as the reference.[4]

Ethical Consideration

Ethical clearance was obtained from the Senate of Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS) with approval number MUHAS-REC-08-2018-50. Managers of selected blood donation centers granted permission to conduct the study. Study participants provided written consent. Confidentiality of the study participants was ensured by using codes instead of their personal names.

RESULTS

Socio-Demographic Characteristics of Study Participants

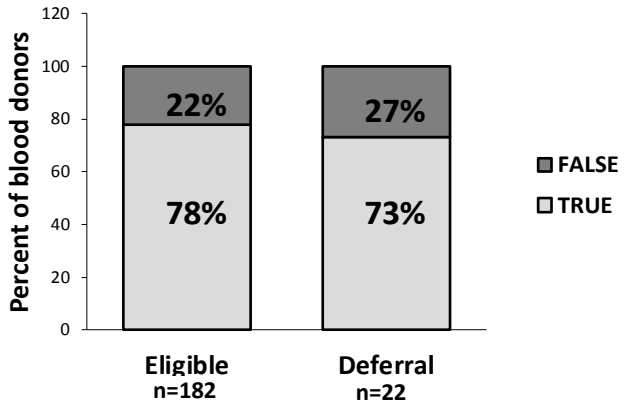
A total of 204 blood donors participated in this study. The median age was 30 years (IQR: 20-39). Males contributed 73.0% of the study participants. The majority of the participants, 39.2% (80/204), were from MNH, followed by 31.4% (64/204) from Temeke blood transfusion satellite site and 29.4% (60/204) from EZBTC.

The Proportion of False Eligible and Deferred Blood Donors by $CUSO_4$ Gravimetric Method

We compared the performance of the $CuSO_4$ gravimetric method with an automated haematology analyzer as a reference. We found that 182 (89.2%) out of 204 participants were eligible donors, whereas 22 out of 204 participants (10.8%) were deferral donors by the $CuSO_4$ gravimetric method. Of the 182 participants eligible by the $CuSO_4$ gravimetric method, 142 (78%) qualified by the automated haematology analyzer, whereas the remaining 40 (22%) were disqualified (Figure 1). Thus, 19.6% (40/204) of the study participants screened by the $CuSO_4$ gravimetric method were falsely eligible.

Furthermore, we observed that 16 out of 22 (73%) of the deferral blood donors were disqualified by both the $CuSO_4$ method and automated haematology analyser, whereas the remaining 6 out of 22 (27%) who were disqualified by $CuSO_4$ gravimetric method were qualified by the automated haematology analyser (Figure 1). These results indicate that 2.9% (6/204) of the blood donors screened by the $CuSO_4$ gravimetric method were false deferrals. Further analysis regarding the participants' demographic characteristics revealed that the proportions of male donors who were falsely eligible and falsely deferred were 55% (22/40) and 66.7% (4/6), respectively (Table 1). In addition, we observed that among the false eligible blood donors, 37.5% (15/40) were aged 21- 30 years, while among false deferred blood donors, the majority, 66.7 % (4/6) were aged 18 to 20 years. (Table 1)

FIGURE 1: Proportions of True and False Eligible and Deferral Donors by CuSO4 Method



Donors identified as eligible (n=182) and deferral (n=22) by the CuSO4 method were analysed for haemoglobin using the automated haematology analyser as the gold standard. The proportions of true and false eligible and deferral donors are indicated.

TABLE 1: Demographic Characteristics of False Eligible and Deferred Blood Donors

Variable	Deferred by $CuSO_4$ n = 22	Falsely Deferred Blood Donors n = 6 (%)	Eligible Blood Donors by $CuSO_4$ n = 182	False Eligible Blood Donors n= 40 (%)
Sex				
Male (55.0)	5	4 (66.7)	144	22
Female (45.0)	17	2 (33.3)	38	18
Age group				
18-20	18	4 (66.7)	40	7 (17.5)
21-30 (37.5)	1	0 (0.0)	48	15
31-40	1	1 (16.6)	53	8 (20.0)
41-50	1	1 (16.6)	27	7 (17.5)
> 50	1	0 (0.0)	14	3 (7.5)

Comparison of $CuSO_4$ and HemoCue Methods

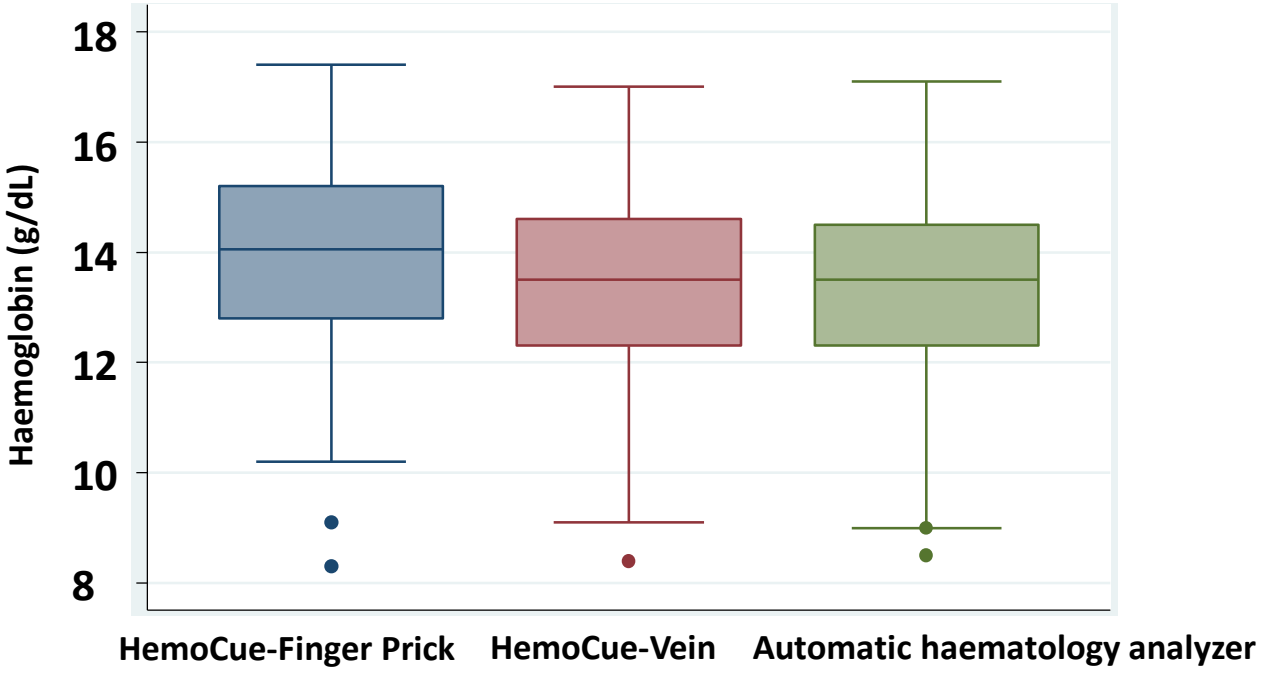
We then compared the performance of $CuSO_4$ and HemoCue methods with the automated haematology analyzer as reference. We observed that the sensitivity and specificity of $CuSO_4$ gravimetric method were 95.9 % and 28.6%, respectively, and the Kappa agreement was 0.1, suggesting a slight agreement (Table 2). In contrast, the HemoCue method had higher sensitivity and specificity of 98.6% and 62.5 %, respectively, and Kappa agreement of 0.63, suggesting a substantial agreement (Table 2). Furthermore, the positive predictive values (PPV) for $CuSO_4$ and HemoCue methods were 78.0% and 87.4%, and the negative predictive values were 72.7% and 94.6%, respectively (Table 2).

Correlation of Haemoglobin from Venous and Finger Prick Blood Samples

In the present study in order to investigate whether the difference in the performance of haemoglobin measurement was due to the blood sampling site or the analytical instrument used, we assessed capillary and venous Hb by HemoCue Hb301. We observed that the median Hb estimated from capillary samples was relatively higher (14.1 g/dL) compared to that from venous route (13.5g/dL) (Figure 2a). We performed a linear regression analysis which showed a positive strong correlation (with r = 0.913) between Hb estimate of venous blood and that of finger prick. Through this we determined the prediction formula of the venous blood from the Hb estimate of finger prick through equation of $y = 0.913x + 0.685$ (Figure 2b).

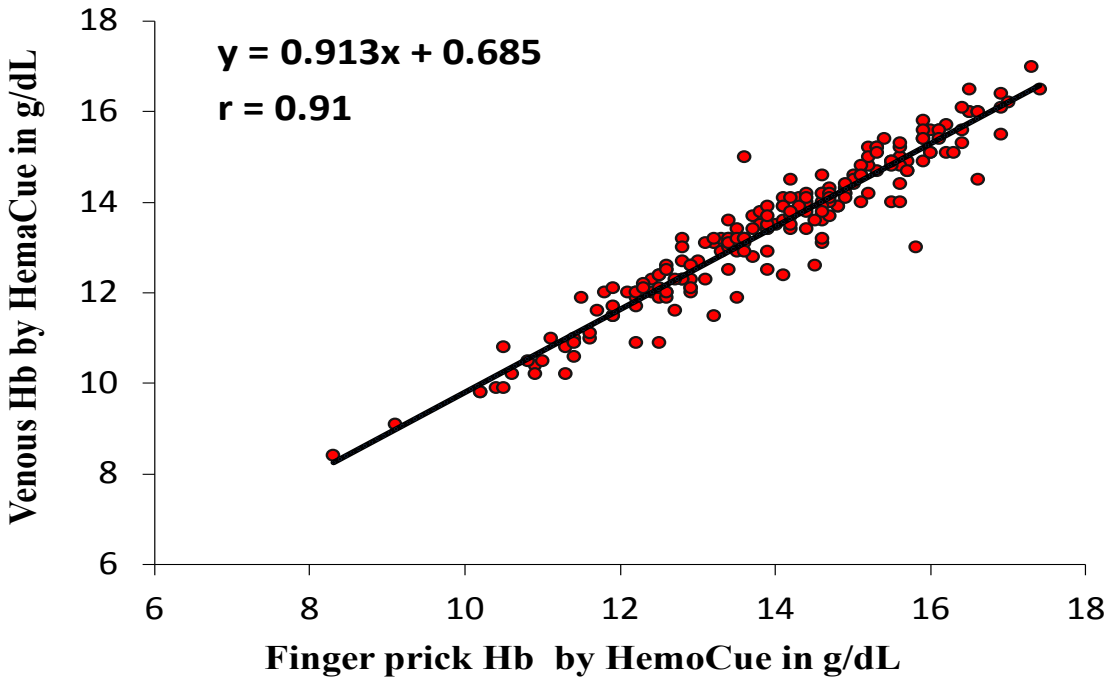
In addition, paired T- test between Hb from finger prick capillary and vein blood samples was performed, whereby the mean difference of Hb between those blood samples from venous route versus finger prick method was 0.53 g/dl and this was statistically significant with *P* value <.001 (Table 3).

FIGURE 2a: Median Quartile and Interquartile Range Using Finger Prick Sample and Venous Sample



The figure depicts the comparison of the distribution of the Hb values estimated by HemoCue method from the finger prick and venous samples.

FIGURE 2b: Correlation of Hb Estimations via Finger Prick and Venous Route as Site of Blood Collection by HemoCue



The figure depicts the correlation of Hb estimation between capillary sample and venipuncture sample by HemoCue

TABLE 2: Performance of $CuSO_4$ and HemoCue Methods Using Automated Haematology Analyser as the Gold standard

	Sensitivity (%)	Specificity (%)	PPV (%)[a]	NPV (%)[b]	Cohen's Kappa (K)
Method					
CUSO4	95.9	28.6	78.0	72.7	0.1
HemoCue	98.6	62.5	87.4	94.6	0.63

[a]Positive predictive value; [b]Negative predictive value.

TABLE 3: Paired T- test Between Haemoglobin From Finger Capillary and Vein Blood Samples

Variable	Standard Error	Standard Deviation	95% CI	T-value	P Value
HemoCue – Finger Prick	0.12	1.70	13.69 - 14.15	15.75	<.001
HemoCue – Vein	0.11	1.62	13.17 - 13.62		
Difference	0.03	0.48	0.46 - 0.59		

DISCUSSION

Accurate estimation of haemoglobin among blood donors is paramount to ensuring the safety of both the donors and recipients. Here, we evaluated the commonly used $CuSO_4$ gravimetric method for haemoglobin estimation in three leading blood donation centers in Dar-es-salaam, Tanzania. Our findings revealed that the method had suboptimal specificity (28.6%), PPV (78.0%), NPV (72.7%), and a slight (0.1) Kappa agreement with automated haematology analyzer; consequently, leading to a high proportion of false eligible donors of 19.6%. On the other hand, the HemoCue method had superior specificity (62.5%), PPV (87.4%), NPV (94.6%), and substantial (0.63) Kappa agreement with automated haematology analyzer.

The present study revealed that 19.6% of blood donors screened by the $CuSO_4$ gravimetric method in the three blood donation centers with the highest number of donors in Dar es Salaam, Tanzania, were falsely eligible. This proportion is higher than that reported in other studies that used the $CuSO_4$ gravimetric method, such as the study by Gupta *et al.* in India that reported a smaller proportion of 3.8% false eligible donors;[20] and that by Guracha *et al.* in Ethiopia at 9.2% of false eligible blood donors.[26] and another study by Chaudhary *et al* found 6.9% of the blood donors were false eligible.[4] Such difference could be due to variations of practice in preparations, quality control measures in the use of $CuSO_4$ gravimetric methods as previously reported to affect the performance.[4, 25] The high proportion of false eligible donors in our study setting increases the risks for donation-induced anemia among donors and inadequate blood transfusions to recipients.

We observed that 2.9% of blood donors screened by $CuSO_4$ gravimetric method and failed were false deferrals. This proportion was higher when compared to the study done by Gupta *et al.* in India, where the proportion of the deferred blood donors was only 1.4%.[20] The high proportion of the false deferred donors in our study may also be due to inadequate quality control checks, as shown previously, to influence the performance of the $CuSO_4$ gravimetric method.[20] The finding suggests that in order to improve the performance of the $CuSO_4$ gravimetric method to estimate haemoglobin among potential blood donors, one needs to improve the frequency and efficiency of the quality control checks.

With regard to $CuSO_4$ gravimetric method sensitivity and specificity, our study showed that the $CuSO_4$ method had high sensitivity. The findings are in line with the sensitivity reported in other previous studies conducted by Sobhy *et al*, Pistorious *et al*, Wilkinson *et al*, Gupta *et al.*, Guracha *et al.*, and Agnihotri *et al*. that reported a sensitivity of 97%, 94%, 95.7%, 98.4%, 94.4%, and 96.55%, respectively.[20, 25-29] In contrast, the specificity of $CuSO_4$ gravimetric method in our study was low compared to that reported by Gupta *et al* (98.8%) and Agnihotri *et al* (74.42%).[20, 25] Furthermore, our study revealed low PPV and NPV for the $CuSO_4$ gravimetric method compared to PPV of 95.8%, 92.3%, and 80% and NPV of 81.0%, 90.7% and 99% reported in previous studies.[20, 28, 30] The difference in PPV between the present study and the previous studies might be due to the non-conformity of the Standard Operating procedure (SOP).

The implication of low PPV and NPV may result in donation-induced iron deficiency (DIID) anemia,[9] and lead to an increased risk of adverse events reporting from the blood donors.[31]

In this study, we observed that the HemoCue method performance was superior to that of $CuSO_4$ gravimetric method. This observation is similar to what has been reported elsewhere.[5, 15, 20, 24, 30, 32] Therefore, these findings suggest HemoCue method may be a reasonable substitute for the $CuSO_4$ gravimetric method in our setting.

CONCLUSION

The present study is the first to reveal the high magnitude of false eligible and deferral blood donors through the $CuSO_4$ gravimetric method in our study settings. This study shows that the $CuSO_4$ method performed poorly compared to the reference method in estimating haemoglobin among blood donors, leading to high levels of false eligibility for donation. On the other hand, the HemoCue method performed better in the same settings. As $CuSO_4$ pentahydrate is still the most affordable method, our findings call for healthcare providers and stakeholders to formulate strategies to improve the screening of blood donors to enhance the quality of donated blood in our setting and other resource-limited settings. More studies are needed for the purpose of quality improvement of this method as it is still the most widely available and most appropriate technique considering the different environments that blood donation is done. Assessment on the frequency of quality control checks and how that affects the accuracy of the haemoglobin estimation could help to find the optimal frequency and points at which quality checks should be done to increase the effectiveness of this method. Investigating the accuracy of the technique with different concentrations of copper sulphate solution is an alternative method that may improve the quality of the technique. In addition, we suggest that in order to save inappropriate deferrals, when $CuSO_4$ method be used for massive screening, and subsequent testing should be done with HemoCue in situations where there is high demand for blood.

Limitation

The present study had a limitation. This study used the gold standard that is only applied to the venous sample due to the fact that Cell dyne analyser need a larger amount of blood and therefore the cell dyne analyser could not be used to test for the finger prick samples.

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