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Research Article

The Level of False Pass and Fail among Prospective Blood Donors as Screened by Copper Sulphate Gravimetric Method at Hossana Blood Bank, Hossana, South Ethiopia

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ABSTRACT

Background: Copper sulphate (CuSO4) gravimetric haemoglobin screening method has been found to erroneously include anaemic people while excluding eligible ones. Determining the rate of false deferral and false pass of prospective blood donors by this obsolete test, the routine screening method in the blood banks of Ethiopia, is the focus of this study.

Methods: In this cross- sectional study capillary and venous blood samples from 422 voluntary donors at Hossana blood bank was analyzed by copper sulphate gravimetric test and automated hematology analyzer. Data was entered in Epi-Data 3.1 and analyzed by SPSS 20. P value less than 0.05 was considered as statistically significant. Agreement between the two methods was determined using the Kappa coefficient.

Results: Overall deferral rate by the reference method was 20.1% (85/422) which was higher than copper sulphate method that also varied by blood collection site: 65 (15.4%) versus 71 (16.8%) for capillary and venous samples, respectively. The copper sulphate method resulted in respective false- pass and - fail rates of 9.2% and 4.5% (capillary blood) and 7.6% and 4.3% (venous blood). Agreement with the reference method was moderate with Kappa value of 0.53, (95% CI, 0.43-0.63, $p<0.01$) using capillary blood and substantial with Kappa value of 0.61, 95% CI (0.51 to 0.70), ($p<0.01$.) when using venous blood.

Conclusion: The high rate of false pass warrants the search for an alternative simple method to replace the old copper sulphate method. Until replaced, as it moderately agrees with the reference method, it can be retained as a primary screening tool by applying strict quality control method.

Keywords: False deferral; Agreement; Copper sulphate gravimetric test; Haemoglobin; Voluntary blood donors; Blood transfusion

INTRODUCTION

Blood transfusion is an indispensable component of health care. It contributes to save millions of lives each year in both routine and emergency situations [1]. In Ethiopia proportion of voluntary blood donation increased from 10% in 2012 to 92.1% in 2014 achieving the WHO regional target of 80% voluntary blood donation [2]. The primary responsibility of a blood transfusion service (BTS) is to provide a safe, sufficient and timely supply of blood and blood products. In fulfilling this responsibility, the BTS should ensure that the act of blood donation is safe and causes no harm to the donor. The pre-donation assessment of donor haemoglobin remains the best approach as there are no rapid, simple and direct bedside methods for determining iron status. The aim of haemoglobin screening is to ensure that the prospective donor is not anaemic [2, 3]. The copper sulphate (CuSO4) specific gravity method is accepted method for mass screening of blood donors. This method was described in 1945 by Phillips et al. for determining the specific gravity of whole blood and plasma [4, 5]. In the copper sulphate method a drop of whole blood is allowed to fall in to the copper sulphate solution with specific gravities of 1.053 and 1.055 to screen female and male donors, respectively. The whole blood maintains its own density, for approximately 15

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seconds. If the drop of blood is denser than the specific gravity of copper sulphate it will sink indicating that the donor's haemoglobin level is acceptable while floating determines rejection. The method does not give quantitative result, has subjective end point, and is difficult to quality control making its utility questionable [6, 7]. Temporary deferral had negative impact on blood donor return rates. Inappropriate rejection of eligible donors affects blood supply in future since once deferred, many donors do not return to attempt another donation [8-14]. In a country where the total amount of collected units per annum is far below of what is expected for the population size, there is a need to estimate the rates of false deferral/pass and take appropriate measures to minimize it. This study is a first attempt in this regard.

METHODS

Study setting and population

This prospective cross sectional study which was conducted from April to June 2017 enrolled 422 blood donors in Hossana town in Southern part of Ethiopia. The blood bank collects 3565 units per year. Copper sulphate gravimetric method using capillary blood is the method of donor haemoglobin screening in the blood bank like any other blood banks in the country. Voluntary blood donors, who were willing to participate and pass other donor selection criteria like age limit, blood pressure, pulse , temperature, weight, were included in this study.

Measurements

Capillary blood was collected following standard operating procedures (SOPs) from ring finger using sterile lancet. The first drop of blood was wiped off and freely flowing capillary blood collected using capillary tube. Four millilitre of venous blood sample was collected in EDTA tube after obtaining informed verbal consent. To evaluate the effect of sampling site variation both capillary and venous blood samples were tested using copper sulphate solution with specific gravity of 1.053 (equivalent to Hb concentration of ≥12.5 g/dl, cut off for female) and 1.055 (equivalent to Hb concentration of ≥ 13.5 g/dl, cut off for male). Donors were classified as pass or fail based on sinking or floating of the sample respectively. The specific gravity measurements are carried out by letting drop of whole blood approximately from 1cm above the surface of the solution fall in to a copper sulphate. The momentum of the droplet takes it to 1-2cm below the surface within 5 seconds. The movement of the drop over the next 10 seconds is observed. If it continues to fall; the hemoglobin level is judged to be above 12.5g/dl or 13.5g/dl. If it rises, the hemoglobin level is taken as less than 12.5g/dl or 13.5g/dl.The venous blood sample was further analysed by automated haematology analyser (Cell-dyn 1800, as a reference method) within 8 hours. Cell dyne 1800 enumerates blood cells by impedance principle and measures Hb using spectrophetometric technique, using modified methemoglobin reagent. During Hb measurement lyse reagent lyses the diluted red blood cells and convert the released hemoglobin to a chromogen, then zero or blank reading is first obtained to provide a reference to which the sample signal is compared. A low energy light emitting diode (LED) is used as a light source and shines through the Hb flow cell and 540 nm narrow band width filter and on to a photo detector. The Hb concentration is directly proportional to the absorbance of the sample. Reference and sample readings are compared to determine the Hb concentration of the sample and result is displayed as Hb in(g/dl) of whole blood. Three level commercial controls are used to check the quality of the analyser.

Statistical analysis

The data obtained from the two tests was entered in Epi-Data version 3.1 and analysed using SPSS Version 20. The specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) of copper sulphate donor screening test was calculated. Agreement between the two methods was determined using Kappa. P-Value <0.05 was considered statistically significant.

RESULTS

Of the 422 voluntary blood donors, 217 (51.4%) were females. The age range was between 18-63 years with a median of 21 years. Majority 311(73.7%) were between the age range of 18-24 years. Large proportion of the donors, 293 (69.4%), were first time donors [Table 1].

Table 1: Demographic characteristics and donation status of study participants at Hossana Blood bank, South Ethiopia, April-June, 2017 (n=422).

Variable	N (%)
Sex	
Male	205 (48.6)
Female	217 (51.4)
Age (in years)	
18-24	311 (73.7)
25-44	102 (24.2)
>44	9 (2.1)
Donation Status	
First time donation	293 (69.4)
Repeat donation	129 (30.6)

The overall deferral rate, as determined by the reference method using the recommended Hb cut off values of 12.5 gm/dl for females and 13.5 gm/dl for males was 20.1% (85/422). As shown in [Table 2], the deferral rate was higher among female donors 58 (68.2%) as compared to male donors 27(31.8%) and among those aged 18-24 years, 82.4% (70/85).

The rate was also higher in first time donors 52(61.2%) compared to repeat donors 33 (38.8%) [Table 2].

Table 2: Rate of pass/fail based on demographic characteristics and donation status as determined by the reference method (Cell-Dyn 1800) at Hossana Blood bank, South Ethiopia, April-June, 2017 (n-422).

Variable	Pass	Fail	Total
Sex			
Male	178(52.8%)	27(31.8%)	205(48.6%)
Female	159(47.2%)	58(68.2%)	217(51.4%)
Total	337	85	422
Age (years)			
18-24	241(71.5%)	70 (82.4%)	311 (73.7%)
25-44	89 (26.4%)	13 (15.3%)	102 (24.2%)
>44	7 (2.1%)	2 (2.4%)	9 (2.1%)
Total	337	85	422
Donation Status			

First time donation	241(71.5%)	52(61.2%)	293(69.4%)
Repeat donation	96(28.5%)	33(38.8%)	129(30.6%)
Total	337	85	422

Comparison of the pass and fail rate of copper sulphate donor screening test using both capillary and venous blood sample against the reference method (Cell-Dyn 1800) is summarized in Table3. The cut off value recommended by WHO was haemoglobin level of not less than 12.0 g/dl for females and not less than 13.0 g/dl for males as the threshold [6]. While 12.5 gm/dl for females and 13.5 gm/dl for males to determine pass/fail status are used in Ethiopia. As shown in the table, among those with Hb value less than 12.5 gm/dl, none were rated as "Pass" by the reference method while 25 and 18 of prospective donors in this category were rated as "Pass" by CuSo4 method using capillary and venous blood samples, respectively. The overall deferral by blood collection site also varies; 65 (15.4%) versus 71 (16.8%) for capillary and venous samples, respectively which is lower than the 85 (20.1%) deferral by the reference method.

Table 3: Deferral rates of donors by Haemoglobin categories determined by reference method (Cell-Dyn 1800) and CuSo4 screening method at Hossana Blood bank, South Ethiopia, April-June, 2017 (n-422).

Haemoglobin value (in gm/dl)	True pass and fail (by reference method)		CuSo4 using capillary blood sample		CuSo4 using venous blood sample	
	Pass	Fail	Pass	Fail	Pass	Fail
<9.5	0	5	0	5	0	5
9.5-10.9	0	16	4	12	3	13
11.0-12.4	0	44	21	23	15	29
12.5-13.9	101	20	96	25	97	24
14.0-15.9	153	0	153	0	153	0
16.0-17.0	56	0	56	0	56	0
>17.0	27	0	27	0	27	0
Total	337	85	357	65	351	71

The performance characteristics of the copper sulphate screening method were evaluated against the Reference method (Cell-Dyn 1800) using both capillary and venous blood samples. Accordingly, as shown in Table 4, the copper sulphate screening test using capillary blood sample inappropriately passed 39/422 (9.2%) donors, while 19/422 (4.5%) of donors were falsely deferred. When using venous blood sample, the copper sulphate screening test inappropriately passed 32/422 (7.6%) donors and falsely deferred 18/422 (4.3%) donors.

Table 4: Performance characteristic of CuSo4 donor screening test against Cell-Dyn 1800 automated haematology analyser at Hossana Blood bank, South Ethiopia, April -June, 2017.

Result	CuSo4 using capillary blood	CuSo4 using venous blood
True positive	318	319
True negative	46	53
False positive	39	32
False negative	19	18
Sensitivity	94.40%	94.70%
Specificity	54.10%	62.40%
PPV	89.10%	90.90%
NPV	70.80%	74.60%

The copper sulphate screening test using capillary blood sample had sensitivity of 94.4%, specificity 54.1%, PPV 89.1% and NPV 70.8%, while using venous blood sample it had sensitivity of 94.7%, specificity 62.4%, PPV 90.9% and NPV 74.6%.

Kappa coefficient was calculated to determine the level of agreement between the copper sulphate and the reference method. The cut off proposed by Landis and Koch as standards for strength of agreement of Kappa coefficient, was used in this study: < 0.00 = poor; 0.00-0.20 = slight; 0.21-0.40 = fair; 0.41-0.60 = moderate; 0.61-0.80 = substantial, 0.81-1.00 almost perfect [15]. Accordingly, the copper sulphate donor screening test using capillary blood sample was in "moderate" level of agreement with the reference method: Kappa (0.53), 95% CI (0.43 to 0.63), p<0.01. Using venous blood sample it was in "substantial" level of agreement with the haematology cell analyser: Kappa (0.61), 95% CI (0.51 to 0.70), p<0.01.

Then we tried to see if any of the factors (sex, age, and previous donation status) could affect the rate of deferrals or pass by the copper sulphate methods by sites of blood sample collection. As shown in Table 5, status of pervious donation and Age has no statistically significant association for both capillary and venous blood samples. Females were 3.75 times more likely to fail the copper sulphate test using venous blood sample than males, while they were 2.67 times more likely to fail the test when using capillary blood.

Table 5: Comparison of deferral rates of donors by age, sex and frequency of previous donation as determined by reference method (Cell-Dyn 1800) and CuSo4 screening method at Hossana Blood bank, South Ethiopia, April-June, 2017 (n-422).

		Capillary blood CuSo4 test		Venous blood CuSo4 test	
Variables	frequency	AOR (95% CI)	P-value	AOR (95% CI)	P-value
Gender					
Male	205(48.6%)	1		1	
Female	217(51.4%)	2.672(1.450-4.923)	0.002	3.745(2.001-7.008)	0.01
Age(years)					
18-24	311(73.7%)	1		1	
25-44	102(24.2%)	0.468(0.199-1.096)	0.08	0.464(0.198-1.090)	0.078
>44	9(2.1%)	0.531(0.199-1.096)	0.55	0.465(0.056-3.894)	0.48
Previous donation					
First time	293(69.4%)	1		1	
Repeat	129(30.6%)	1.191(0.672-2.110)	0.557	1.375(0.790-2.394)	0.261

DISCUSSION

This study determined the level of false deferral and false pass of prospective blood donors screened by Copper Sulphate Gravimetric method at Hossana Blood Bank, South Ethiopia, using Cell-Dyn 1800 as a reference method. The study demonstrated CuSo4 method using capillary and venous blood sample inappropriately passed 9.2% and 7.6% of donors, respectively; of which majority

(75%) were within 1.0g/dl of threshold against the reference values. This finding was slightly higher than the observation made by Malukani et al. (5%) in India using Sysmex kx 21 as a reference method [16]. The false pass rate in our study using venous blood was higher than the observation made by Tondon et al. (6.9%) in India using ABX micros 60 as a reference method [17].

Other studies have also demonstrated high rates of inappropriate pass by the copper sulphate method. For instance, James et al. in UK reported 16.4% using Bekman Coulter as a reference method [14]. Nadarajan et al. (21.3%) in Malaysia using ABX Vega retic [18] and Gomez-Simon et al. (83%) in Spain using Coulter Electronics [19] also observed more inappropriate pass by the CuSo4 method. On the other hand, significantly low pass rate compared to the current study was observed by Gupta et al. (3.8%) in India using ERMA PCE 210 cell analyzer [20], Lewis SM (0.9%) in UK using Sysmex KX 21 [21]. The observed differences between the various studies can be explained by the subjectivity of end point determination by the copper sulphate method, though instrument variations cannot be totally excluded.

In this study, the copper sulphate method when using capillary blood sample tends to give more false pass rate as compared to venous sample, this difference might be due to higher haemoglobin value in capillary blood [19,22,23]. The CuSo4 gravimetric method has also been found to give inappropriate failure and approximately 50% of deferred donors could be recovered by testing with different method [24]. The current study revealed that copper sulphate method by using capillary and venous blood sample inappropriately deferred 4.5% and 4.3% of donors, respectively. Finding of this study using capillary blood sample was almost consistent with that of Lewis SM (5.2%) in UK using sysmex KX 21 as reference method [21]. But Mathur et al. (37.81%) [25], and Sawnt et al. (29%) both from India using coulter counter as reference method [26] found more false deferral rate as compared with our finding. This difference might be due to using donors who fail the primary copper sulphate screening test as their study participants. Slightly higher false deferral rates was reported by James et al. (8.1%) in UK[14]. On the other hand, Malukani et al. (3%) [16], Tondon et al. (1.3%)[17], Gupta et al.. (1.4%) all from India [20] and Gomez-Simon et al. (1%) in Spain [19] observed less false deferral rates, again underscoring the subjectivity of the old copper sulphate method of donor screening.The calculated sensitivity of copper sulphate method for capillary and venous blood sample in the current study was 94.4% and 95%, respectively. This finding is slightly lower than that of Malukani et al. (96.36%) [16], Gupta et al. (96.8%) [20], and Tondon et al.. (98.8%) [17]. Whereas Kim et al. (81.8%) in Korea using Sysmex KX-21N as a reference method found low level of sensitivity[24].Specificity of copper sulphate gravimetric method when using capillary and venous blood sample was 54.1% and 62.4%, respectively. Kim et al. (95.2%) in Korea using Sysmex KX-21N [24] and Malukani et al. (71.43%) in India using Sysmex kx 21 as a reference method [16] observed high level of specificity, respectively as compared to ours. Variability in specificity based on the source of blood sample is reflected by the observation that the specificity observed in the current study (54.1%) parallels with Gupta et al. (55.1%) for capillary blood but lower when using venous blood (62.4% vs 55.1%) [20]. Lower specificity was also reported by others [17]. An earlier study from Ethiopia by Semie et al. [27] observed similar level of sensitivity and high level of specificity as compared to our finding. This difference might be due to using different strength of copper sulphate solution (Sp. G. 1.044 and Sp.G.1.049) and different cut off value for haemoglobin (<8g/dl and <11.0g/dl) were used by Semie et al. The current study used copper sulphate concentrations and Hb cut offs routinely practiced in all blood banks of Ethiopia.

Based on Landis and Koch's standard [15], the copper sulphate donor screening test using capillary blood sample was in moderate level of agreement with the reference method: Kappa 0.53, 95% CI (0.43 to 0.63), p<0.01. Using venous blood sample, it was in substantial level of agreement with the automated haematology analyser: Kappa 0.61, 95% CI (0.51 to 0.70), p<0.01. Considering the routine practice of using capillary blood for donor screening using the copper sulphate method in the blood bank, and the Ethiopian Federal Ministry of Health's plan of recruiting large number of voluntary donors, the moderate level of agreement observed between the test and the reference method is of concern. As deferral may have a negative impact on donor satisfaction [13], effort has to be made to have reliable method of screening to maximize donors' satisfaction and maintain them in the donation program. Of interest, in the current study females were 3.75 times (using venous blood sample) and 2.67 times (using capillary blood sample) more likely to fail the copper sulphate screening test than males due to low haemoglobin level. The most important cause might be low iron stores in women due to menstruation and pregnancy. Mast et al. in United States (OR 11.27 with 95% CI of 10.09 to 12.59) [28] and Ngoma et al. in Japan (OR 35.48 with 95% CI 27.74 to45.38) [29] observed higher odds for deferral due to low hemoglobin in females compared to our finding. The use of same haemoglobin (12.5 g/dl) cut-off values by Mast et al. [28] to qualify male and female donors despite differences in normal range values for men and women might explain the observed variations. Overall when we see some of the discrepancies interims of sensitivity, specificity or magnitude of false deferral and pass among the many reports mentioned, the contribution of differences in automated instruments or principle in Hb determination cannot be excluded, and it would have been good to do the test with two or more variety of instruments, which this study didn't do and we had also limitation to test a rapid point of care Hb devises and compare its performance for potential use as screening test. Regardless, this report revealed the magnitude of false deferral and pass of blood donors in our context and tried to compare where we stand among different countries. Such information should be passed and circulate among scholars and different stakeholders who are working to improve the blood donation pool and quality, especially among developing nations.

CONCLUSION

This study has demonstrated high rate of false pass using copper sulphate gravimetric method, hence ineligible donors are being recruited to donate blood which would have negative impact on their health as well as future donation. On the other hand, the false deferral in the donor population is dominated by the young and unlike many sites in Ethiopia by female donors, this will have implications in retaining voluntary blood donors in the program. In a country where the annual voluntarily donated blood unit is far below the target, any factor compromising the donors retention have to be dealt, there for it is timely to introduce alternative Hb screening methods.

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