

Use of the copper sulphate method as a tool for screening for anaemia in field antenatal clinics

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Abstract

Objective : To assess the feasibility of using the copper sulphate method as a screening procedure to detect anaemia in field antenatal clinics

Methodology : A total of 311 pregnant women were tested for the presence of anaemia using the copper sulphate method by trained Public Health Midwives. The validity of the method was assessed using the HaemoCue method. Observations of the anaemia status made by the midwife and a technician were compared to assess the ability of the midwife to assess the anaemia status.

Results: The sensitivity of the copper sulphate method for different cut off points; i.e. 9 g/dl, 10 g/dl and 11 g/dl were 100%, 95% and 82% respectively. Assessment of inter-observer variation between the midwife and the technician indicated a good level of agreement ($k = 78.0\%$). The cost of consumables required was lower than for the HemoCue method and the specific gravity of the solution remained unaltered for approximately six months.

Conclusions: The copper sulphate method can be used as a screening test for anaemia among pregnant women in field settings with a satisfactory level of validity and relatively low cost. Midwives could be trained to carry out this test with minimal inputs.

Key words: Anaemia, screening test, pregnant women, field clinics

Introduction

Anaemia is one of the commonest nutritional deficiency diseases in the world today. Almost half the women in the developing world are anaemic, and among pregnant women this figure rises closer to 60%¹. Anaemia is commonly detected by measuring haemoglobin or by determining the haematocrit value. According to the World Health Organisation (WHO) Scientific Working Group anaemia among pregnant

women living at sea level, is indicated by a haemoglobin (Hb) level below 11g per decilitre².

To minimise negative effects for the mother and the new-born, it is necessary to detect anaemia early and initiate appropriate therapy. Thus, screening for anaemia has to be carried out in all antenatal clinics, those at field level and at institutions. Identifying a suitable method for screening pregnant women for anaemia in field clinics is important if such a programme is to be implemented successfully. Such a method should be valid, simple to operate, require minimal infrastructure facilities and be of low cost.

A method recommended for use in field settings is the 'HemoCue' method where whole blood is converted to azide methaemoglobin in a disposable, chemically treated cuvette and the Hb level measured photometrically. This method had shown good sensitivity and specificity³. However, the cost of the instrument is high and one pre-packed disposable cuvette has to be used per sample.

The relatively simple copper sulphate method where the Hb level is assessed by the sinking / floating status of a drop of whole blood in a copper sulphate solution of a known specific gravity has been considered as a method that could be used at field clinics⁴. In Sri Lanka, where reported studies indicate a high prevalence of anaemia in pregnancy^{5,6}, the consultative group that developed the national strategy and broad plan of action for prevention and control of anaemia in pregnancy recommended that a feasibility study be undertaken on the use of the copper sulphate method to screen pregnant women for anaemia⁷.

This report presents the results of an assessment of the feasibility of using the copper sulphate (CuSO₄) method, in field settings. The validity of this method was assessed with reference to the HaemoCue method

Materials and methods

All pregnant women seen at the antenatal clinics in three Divisional Director of Health Services areas in the Colombo district and an antenatal clinic held within the Colombo Municipal Council area within a two week period, were identified as the study population. A total of 311 pregnant women who gave informed consent was included in the study.

The copper sulphate (CuSO₄) stock solution of specific gravity 1.1000, was prepared in the laboratory using 159.0 g of analar CuSO₄.5H₂O⁴. The specific gravity was confirmed by pycnometric analysis. Facilities required for the preparation of the CuSO₄ solution included an analytical weighing scale, an

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instrument to check the specific gravity and tight screw bottles to store the solution. To discriminate the Hb level of 11mg/dl, 48.2ml of this solution was diluted in 100ml of distilled water and a solution with a specific gravity of 1.049 was prepared⁸. This solution was used to carry out a pilot test among 42 volunteers. A sample of the solution was kept in the laboratory and the specific gravity was checked every month, for a six month period using a pycnometer. The technique of preparation was simple and the distribution of the prepared solution to the field clinics did not need any special facilities.

All 30 Public Health Midwives (PHMM) and four Public Health Nursing Sisters (PHNSS) in the selected antenatal clinics were trained for one day in the use of copper sulphate method. They were trained to perform the technique and were provided with written instructions.

To carry out the test, the midwife pricked the left middle finger of each pregnant woman, using a sterile disposable lancet. The woman was instructed to put the drop of blood into the CuSO_4 solution from a height of about 1cm from the surface of the solution kept in a beaker. The status of the drop of blood in a copper sulphate solution was assessed by the midwife in the following manner: floating - when the blood was floating in the solution, sinking - when the drop was falling to the bottom and neutral - when the drop stayed in solution for 10-20 seconds. This implied that a blood droplet had a lower (floating), greater (sinking) or equal (neutral) specific gravity when compared to the specific gravity of the copper sulphate solution.

Independent of the observation made by the midwife, a technician assessed the anaemia status by observing the status of blood drop in the copper sulphate solution. The midwife and technician marked their observations on separate records. Inter-observer variability was assessed using Kappa index (k).

To carry out the HaemoCue method, another technician obtained a separate drop of blood from the same prick and haemoglobin was assessed using the HaemoCue method, using the following procedure. A drop (10 microlitres) of blood was placed in a disposable plastic cuvette, which was then placed in a special photometer, when the haemoglobin value was displayed digitally within 45 seconds. The photometer was standardised daily, by using a 'control cuvette'.

The cost per test was assessed only in terms of the cost of the consumables required for the preparation of the solution. Even though this process has limitations in the assessment of the actual cost, this method of estimating cost can be justified as the cost of other

equipment, reagents, capital equipment and other infrastructure facilities for this test are minimal.

Results

The comparison of the haemoglobin estimates from the HaemoCue method and the anaemia status as assessed by the copper sulphate are presented in Table 1. The results show that 40.8% of blood drops were 'floating' in the solution indicating that in this group, the Hb concentration was below 11gms/dl. According to the HaemoCue method the percentage of women with a Hb value of less than 11 gms/dl was 43.7%.

Table 01 Comparison of haemoglobin estimates from HemoCue method and Copper Sulphate method

HaemoCue method Hb concentration (g%)	Copper sulphate method			Total
	Floating	Sinking	Neutral	
6-	3 (100.0%)	0 (0.0%)	0 (0.0%)	3 (1.0%)
7-	3 (100.0%)	0 (0.0%)	0 (0.0%)	3 (1.0%)
8-	4 (100.0%)	0 (0.0%)	0 (0.0%)	4 (1.3%)
9-	49 (94.2%)	3 (5.8%)	0 (0.0%)	52 (16.9%)
10-	52 (71.2%)	13 (17.8%)	8 (11.0%)	73 (23.5%)
11-	14 (17.9%)	56 (71.8%)	8 (10.3%)	78 (25.1%)
12-	2 (2.6%)	74 (97.4%)	0 (0.0%)	76 (24.4%)
13-	0 (0.0%)	19 (100.0%)	0 (0.0%)	19 (6.1%)
14-	0 (0.0%)	3 (100.0%)	0 (0.0%)	3 (1.0%)
Total	127(40.8%)	168(54.0%)	16 (5.1%)	311 (100.0%)

The sensitivity, specificity, positive and negative predictive values for detection of anaemia using the CuSO_4 method was carried out for different levels of Hb "cut off" points as assessed by the HaemoCue method (Table 2). The ability of the CuSO_4 method to detect anaemia i.e. sensitivity, was 100% at a Hb level of 9g/dl and this percentage declined as the Hb value increased. The sensitivity of the method at a Hb level of 11 g/dl was 82.2% The highest positive predictive value of 87.4% was at a Hb value of 11.0g/dl indicating the high probability of those identified as anaemic, as being anaemic.

Table 2 Sensitivity, specificity, positive and negative predictive values (PPV, NPV) for diagnosing anaemia at different 'cut off points' of Hb concentration

Cut-off Hb (g/dl)	Sensitivity (% CI)	Specificity (% CI)	PPV (% CI)	NPV (% CI)
< 9	100.0 (65.5,100)	61.1 (55.3,66.6)	7.9 (4.1,14.4)	100.0 (97.5,100)
< 10	95.2 (85.6,98.7)	72.7 (65.6,78.7)	46.5 (37.6,55.5)	98.4 (94.9,99.6)
< 11	82.2 (74.5,88.1)	90.9 (85.4,94.5)	87.4 (80.1,92.4)	97.0 (81.0,91.3)

The inter-observer variability between the midwife and the technician indicated a percentage agreement of 85.7% in identifying a woman as 'anaemic' while the comparable percentage for classifying women as 'not anaemic' was 86.7% (Table 3). The measure of agreement was calculated by using the kappa index (k). The value of k was 0.78, indicated a good strength of agreement.

Table 3 Comparison of recording the "status" of the blood drops in the copper sulphate method by Medical Laboratory Technologist (MLT) and Public Health Midwife

	Public Health Midwife		
	Floating	Sinking	Neutral
Floating	84 (85.7%)	7 (7.7%)	0 (0.0%)
Sinking	14 (12.4%)	98 (86.75)	1 (0.9%)
Neutral	0 (0.0%)	2 (28.6%)	5 (71.4%)
Total	98 (46.4%)	107 (50.7%)	6 (2.8%)

The repeated measurements of the specific gravity of the solution over a six month period did not show any variation when assessed by pycnometric analysis. It was observed that one hundred millilitres (100ml) of the CuSO_4 solution can be used to check the Hb levels of 50 blood samples, giving the cost of consumables per test to be 40 cents. For the HaemoCue test, the cost of a micro cuvette is 50 rupees and one cuvette has to be used for one blood sample. Comparison of the important characteristics of the two tests is presented in Table 4.

Table -04 Comparison of copper sulphate and HaemoCue methods for assessing anaemia

Method	Copper sulphate	HaemoCue
General category	qualitative	Quantitative
Required electricity	No	Yes
Chemical	Yes	Yes
Level of skills	Low	Medium
Complexity of operation	Low	Low
Accuracy	good	Highly acceptable
Time to obtain results	1 minute	30 seconds
Initial cost of instruments	None	Rs. 35,000
Recurrent cost per test	40 cents	Rs. 50

Discussion

This study demonstrated that in detecting anaemia among pregnant women, CuSO_4 method had a sensitivity of 82.2 % and a specificity of 90.9% when carried out at field clinics, by trained midwives. Previous studies evaluating the diagnostic accuracy of the copper sulphate method have reported it to be sufficiently reliable when used in the hospital^{9,10}. Compared to the Talquist method that was used earlier in the field clinics in Sri Lanka, this method has a higher degree of sensitivity¹¹.

The 'reference' used for assessment of validity in the present study was the 'HemoCue' method, which has been shown to be as good as, if not better than the cyanmethaemoglobin method which is recommended by the Committee for Standardisation in Haematology^{3,10}.

The feasibility of carrying out the test at field clinics in Sri Lanka depends on the ability to train the field staff i.e. the PHMM, with minimal input, capability of the PHM to carry out the test in the field setting, availability of laboratory requirement to carry out the test and the cost per test. The satisfactory level of agreement shown between the observations made by midwife and the technician indicated that midwives could be trained to carry out this assessment, following a brief training programme.

The requirements to carry out the test in the field clinics were minimal and they included beakers to keep the solution while testing and a tight screwed bottle to keep the solution. Facilities required to prepare the solution was minimal and the solution could be prepared at a Base Hospital / District Hospital with minimal laboratory facilities.

Even though the inability to obtain a quantitative estimate of the Hb level poses a limitation, the satisfactory level of validity, feasibility of implementation at field level and the low cost makes this method an appropriate screening procedure to detect anaemia among pregnant women in field settings.

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