MANUSCRIPT

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Title : Screening for anaemia in pregnant women at field antenatal clinics with copper sulphate method.

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Abstract

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

Methodology : A total of 311 pregnant women attending field antenatal clinics were tested for the presence of anaemia using the copper sulphate method , on a sample of blood obtained from a finger prick. The Public Health Midwives were trained to use this method where the anaemia status is determined by observing the floating/sinking status of the drop of blood in the copper sulphate solution, when allowed to drop directly to the solution. Validity of the method was assessed using the HaemoCue method, conducted by technicians. Observations of the anaemia status made by the midwife and a technician were compared to assess the ability of the Midwife to make a correct assessment of 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. Testing for the inter observer variation between the midwife and the technician indicated good level of agreement (kappa index of 78.0%). The cost of consumables required for the test was much lower than the HemoCue method and the specific gravity of the solution when properly prepared and stored was approximately 6 months.

Conclusions: The study demonstrated that 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. Midwifes could be trained to carry out this test with minimal inputs. Possibility of using the copper sulphate solution prepared at a laboratory at a Base Hospital for a period of approximately six months, is an added advantage for using this method in field clinics.

Key words: Anaemia, Screening test, and 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 a major contributory cause in 20% to 40% of the half million maternal deaths occurring globally every year[1]. It also increases the incidence of low birth weight, anaemia and protein energy malnutrition in infants[2]. 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 decilitrere[3].

Early detection of anaemia in pregnancy and initiating appropriate therapy aims at reducing the negative health effects to both the mother and the new born. Hence, screening for anaemia has to be carried out in all antenatal clinics, those at filed level and at institutions. Thus, identifying a suitable method for screening pregnant women for anaemia in field clinics is an important consideration. Such a method should be valid, simple to operate, require 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 Haemoglobin level measured photometrically at a specified wavelength (565 nm). The haemoglobin value is displayed digitally. This method had showed good sensitivity and specificity[4,5,6]. However, the cost of the instrument is high 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 clinics[4,7]. In Sri Lanka, where reported studies indicate a high prevalence of anaemia in pregnancy[8,9,10,11]. 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 copper sulphate method to screen pregnant women for anaemia[12].

This report presents the results of an assessment of the feasibility of using the copper sulphate ($Cuso_4$) method, in field settings. Validity of this method was assessed with reference to the HaemoCue method .

Materials and methods

All pregnant women seen at the antenatal clinics of three Divisional Directors of Health Services (DDHS) areas in the Colombo district and an antenatal clinic held within the Colombo Municipality within a two week period, were identified as the study population. A total of 311 pregnant women who gave informed consent were recruited into the study.

All 30 Public Health Midwives (PHMM) in the selected antenatal clinics and four Public Health Nursing Sisters (PHNSS) participated in a one day training programme in the use of copper sulphate method. They were briefed about the technique, a practical session held and written instructions distributed.

The copper sulphate stock solution of specific gravity 1.1000, was prepared in the laboratory using 159.0g of analar CuSo4.5H2O [7]. The specific gravity was confirmed by pyknometric analysis. The facilities required for the preparation of the CuSo4 solution included an analytical weighing scale, an instrument to check the specific gravity of the prepared solution, and tight screw bottles to store the solution to prevent evaporation. The technique of preparation was simple and the distribution of the prepared solution to the filed clinics did not need any special facilities.

To discriminate the haemoglobon level of 11mg/dl, 48.2ml of this solution was diluted in 100ml and a solution with a specific gravity of 1.049 was prepared[13]. 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 pyknometer.

To carry out the test, the midwife made a 'prick' on 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 copper sulphate solution from a height of about 1cm from the surface of the Copper Sulphate 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: f**loating** - when the blood was floating in the solution, s**inking** - when the drop was falling to the bottom and n**eutral** - 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 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 micro litre) of blood was placed in a disposable plastic cuvette, which was then placed in a special photometer and the haemoglobin value was displayed digitally within 45 seconds. The photometer was standardised daily, by using a 'control cuvette'.

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, Hb concentration was below 11gms/dl. According to the HaemoCue method the percentage of women with a Hb value of less that 11 gms/dl was 43.7%.

The sensitivity, specificity, positive and negative predictive values for detection of anaemia using the $Cuso_4$ method for different levels of Hb "cut off" points were studied, in comparison with HaemoCue method. Results are shown in Table 2. The ability of the CuSo4 method to detect anaemia i.e. sensitivity, was 100% at a Hb level of 9gms/dl and this percentage declined as the Hb value increased. The sensitivity of the method at a Hb level of 11 gms/dl was 82.2% The highest positive predictive value of 87.4% was at a Hb value of 11.0g/dl indicating the high probabaility of those identified as anaemic, as being anaemic.

The inter-observer variability between the midwife and the technician indicated a percentage agreement of 85.7% 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 (κ). The value of κ was 0.78, indicated a good strength of agreement.

The repeated measurements of the specific gravity of the solution over a six month period did not show any variation when assessed by pyknometrivc analysis. It was observed that one hundred millilitres (100ml) of the CuSo4 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 5.

Discussion

This study demonstrated that in detecting anaemia among pregnant women, the copper sulphate 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[14,15]. Compared to the Talquist method that was used earlier in the filed clinics in Sri Lanka, this method has a higher degree of sensitivity[16].

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 cyanomethaemoglobin method which is recommended by Committee for standardisation in Haematology[5,16].

The feasibility of carrying out the test at field clinics in Sri Lanka depends on the ability to train the field staff i.e. PHMM, with minimal input, capability of the PHM to carry out the test in the filed setting, availability of laboratory requirements 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 midwifes could be trained to carry out this assessment, following a brief training programme.

The requirements to carry out the test in the filed clinics were minimal and they included beakers to keep the solution while testing and a tight screwed bottle to keep the solution and the facilites 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 inability to obtain a quantitative estimate of the Hb level poses a limitation , the satisfactory level of validity , e feasibility of implementation at filed 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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Table 1

Frequency of haemoglobin concentration in compare to the different methods

HaemoCue method	Copper sulphate method			Total
Hb concentration (g%)	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%)**

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 (85.6,98.7)	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)

(CI = 95% confidence interval)

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

MLT	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%)

Table 4

Comparison of copper sulphate and HaemoCue methods for assessing anaemia

Method	**General category**	**Requires electricity**	**Chemical**	**Level of skill**	**Complexity of operation**	**Accuracy**	**Time t obtain results**
Copper sulphate	Qualitative	No	Yes	Low	Low	Good	1 minu
HaemoCue	Quantitative	Yes	Yes	Medium	Low	Highly acceptable	30 secc