

## Study of Evaluation of Sensitivity and Specificity of Simple Screening Methods for Assessment of Anaemia in Pregnant Women

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### Abstract

**Aims:** To evaluate the method for screening of Anaemia at primary Health centre (PHC) in respect of sensitivity and specificity of various simple screening test for Anaemia, which should be cheap and simple to operate. **Method:** For this study, 100 ANC cases were examined in ANC clinic at Department of Obstetrics and Gynecology, JNMC (Sawangi, Wardha), Maharashtra and ANC clinic, civil hospital of Wardha. The various methods were studied in Central Clinical laboratory of Biochemistry AVBRH, Sawangi. 100 ANC cases were examined for estimation of Hemoglobin by Cyanmethaemoglobin method, Sahli's Haemoglobinmeter and Specific gravity method. **Results:** Sahli's method has sensitivity of 86.20% at 11gm% and hence Sahli's method can be used in ANC clinic and at primary health centre where all the facilities are made available. Copper sulphate has accuracy of 96.55% at 11gm% and can be used in field. **Conclusion:** Copper sulphate and Sahli's method should be exclusively provided at every primary Health Centre.

**Keywords:** Anaemia, Hemoglobin, Sensitivity, Specificity, Pregnancy

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### Introduction

Anaemia has been known since time of Aristotle (384-322 B.C.) but the problem of anaemia in pregnancy has been of perpetual interest since last century all over the world. Anaemia not only is commonest complication met during pregnancy but also is commonest haematological disorder found worldwide especially in India and other developing countries<sup>[1]</sup>. As per WHO estimation 14% pregnant females in developed and 51% pregnant females in developing countries are anaemic. Contribution of Indian pregnant females in this anaemic group is in between 65 to 75%<sup>[2]</sup>. 80% of South Asian maternal deaths occur in India alone<sup>[3]</sup>.

Pregnant women are at special risk of Anaemia due to increased iron requirement, short birth intervals (blood loss) and prolonged lactation (iron loss). Severity of Anaemia varies with the

socioeconomic status of people. Diet habits, age of marriage are some other closely related incriminated factors. Factor such as food, chiefly derived from local ingredients and prevalence of parasitic infection varies. Anaemia increases steadily with decrease in the level of education and in low socioeconomic females although it is not uncommon in high income groups. Socio-economic developments, higher standard of living, better utilization of health care facilities and better education level decreases association between pregnancy and anaemia hence anaemia is less common in developed countries<sup>[4]</sup><sup>[5]</sup>. Therefore the pattern of incidence, severity and the type of anaemia is likely to vary from place to place.

During a single turn of pregnancy, maternal plasma volume gradually expands by approximate 50% i.e. 1000ml. The total RBC mass also increases but only by approximate 25% i.e. 300ml and this starts later in pregnancy. It is not surprising therefore that Hb

and hematocrit levels usually fall during gestation. Anaemia in pregnancy is a hemoglobin (Hb) concentration less than 11gm/dl in first and third trimester and 10.5gm/dl in second trimester [6]. Guidelines of WHO expert group consider less than 11gm/dl haemoglobin during pregnancy as anaemia in pregnancy [7].

Diagnosis of anaemia depends on the measurement of concentration of haemoglobin in the blood. Measurement of Hb concentration is an important procedure in developing countries but little is known about the results obtainable with sample techniques used in primary health centre for monitoring patients under treatment and for screening of anaemia. Estimation of Hb can be done by assessing the color of blood (Since Hb is main pigment), erythrocyte volume fraction and specific gravity of blood. From the present study, it was tried to assess sensitivity and specificity of some common and simple methods of assessment of anaemia which are cheap, easily available and convenient at peripheral level [8].

## Material and methods

The present study was carried out in ANC clinic, Department of Obstetrics and Gynecology, JNMC, Sawangi (M), Wardha and ANC clinic, civil hospital, Wardha and the investigations were carried out at laboratory of biochemistry, central clinical laboratory AVBRH, Sawangi (M), Wardha. 100 ANC cases were examined for estimation of Hb by Cyanmethaemoglobin method, Sahli's Haemoglobinometer and Specific gravity method [8] [9].

### *Cyanmethaemoglobin Method*

In this is colorimetric method Hb is converted into methamoglobin, carboxyhemoglobin and Cyanmethaemoglobin. An aliquot of well mixed whole blood (Hb) is treated with reagent containing potassium cyanide, potassium ferricyanide and potassium dihydrogen phosphate (called Drabkin's solution). The ferricyanide converts ferrous iron to ferric iron thus forming the methemoglobin which is then converted to stable red compound, Cyanmethaemoglobin by the cyanide. Intensity of red color is proportional to Hb concentration and is measured against standard in photoelectric

colorimeter at 540 nm (green filter). A Cyanmethaemoglobin method was taken as a standard method, which has a standard of 60. This standard was taken for comparison.

### *Sahli's Haemoglobinometer (Acid Hematin) Method*

In this method Hb is converted into acid hematin by the action of hydrochloric acid, a stable coloring substance and compared with the unknown standards. The acid hematin solution is further diluted with the acid until its color matches exactly with that of permanent standard of the comparator block. The Hb read directly from the calibration tube. Blood up into 20 mm is drawn in Haemoglobinometer pipette and it is dipped into calibrated Haemoglobinometer tube containing N/10 HCl up to 20 marks. The blood in acid HCl is stirred for some time and it is matched with standard by adding distilled water if needed. The Haemoglobinometer tube has calibration in percentage of Hb or in grams, so a direct reading is obtained.

### *Specific Gravity (Copper Sulphate) Method Principle*

When a drop of whole blood is dropped into a series of  $\text{CuSO}_4$  solution of known specific gravity, the drop will maintain its own density for approximately 15 sec. The density of drop is directly proportional to the amount of Hb in that drop. If the drop is denser than specific gravity of solution, the drop sinks to the bottom, if not, it will float on top.

Stock Copper sulphate solution is diluted with distilled water, so as to make specific gravity of 1.045 for 11 gm%, 1.031 for 8.8gm% and 1.017 for 6.6gm% for 100ml of blood. A blood drop was allowed to fall into copper sulphate solution with the specific gravity equivalent to that of blood with Hb contents of 11gm/dl from about 1cm above the surface within 5 seconds. The movement of the drop over the next 5-10 sec was observed. If it continued to fall, the Hb level was judged more than 11gm/dl and if it is raised the Hb level was taken as less than 11gm/dl. The test was repeated in copper sulphate solution with specific gravity equivalent to haemoglobin level of 8.8 gm/dl and 6.6gm/dl consequently, every sample was categorized to as below 6.6gm/dl between 6.6-8.8gm/dl, 8.8-11gm/dl and above 11gm/dl.

## Results

11gm% is cut off point for anaemia in pregnant women as laid down by WHO expert group. 6.6gm% is considered as very severe anaemia, 6.6-8.8gm% is considered as severe anaemia and 8.8-11gm% is considered as moderate anaemia (Table- 1).

Sensitivity of copper sulphate decreased from 96.5% at 11gm% to 62.5% at 6.6gm%, while it's specificity increased from 71.42% at

11gm% to 93.47% at 6.6gm%. Sensitivity of Sahli's method also decreased from 86.20% at 11gm% to 75% at 6.6gm%, while it's specificity increased from 83.33% at 11gm% to 93.47% at 6.6gm% (Table- 2). Time needed to estimate Hb by Copper sulphate method just 58 seconds hence this was given first rank. Sahli's method required 120 seconds while Cyanmethaemoglobin method required 180 seconds hence given 2<sup>nd</sup> and 3<sup>rd</sup> rank respectively.

**Table -1: Haematological Status Tests (WHO cut off point: 11 gm/dl)**

Method	Cases	For 11 gm% *		For 8.8 gm%**		For 6.6 gm%***	
		Anaemic	Non-Anaemic	Anaemic	Non-Anaemic	Anaemic	Non-Anaemic
Cyanmethoglobin	100	58	42	28	72	08	92
Sahli's Method	100	57	43	28	72	12	88
Copper Sulphate	100	68	32	25	75	11	89

\*Moderate anaemia, \*\*Severe anaemia, \*\*\*Very severe anaemia

**Table- 2: Sensitivity and Specificity for each Test**

Methods	Sensitivity	Specificity
<b>For 11 gm%</b>		
Copper Sulphate	96.55%	71.42%
Sahli's Method	86.20%	83.33%
<b>For 8.8 gm%</b>		
Copper Sulphate	75%	94.44%
Sahli's Method	82.14%	93.05%
<b>For 6.6 gm%</b>		
Copper Sulphate	62.50%	93.47%
Sahli's Method	75%	93.47%

## Discussion

The purpose of this study was to find out an anaemic screening device for use at primary health care level that would be accurate, easy to use and easy to study and that would give immediate results. At primary and sub center health care system level clinical examination is the most common method of diagnosis of anaemia and among clinical methods pallor is the most commonly-used methods [10]. At peripheral level, haemoglobin colour scale, a WHO recommended method for diagnosis of anaemia has sensitivity around 50% to 86% [10] [11]. van den Broek NR in rural clinics for diagnosing anaemia in pregnancy observed that clinical examination of pallor have lower sensitivity but better specificity than Haemoglobin Colour Scale method [5].

In the present study cyanmethaemoglobin method is taken as a standard method and Sahli and Copper sulphate methods were compared with it for sensitivity and specificity. WHO recommended 11gm/dl is taken as a cut off point for detecting anaemia in pregnant mothers [7]. We found that Sahli's method has sensitivity of 86.20% at 11gm% and copper sulphate has sensitivity of 96.52% at 11gm%. Pankaj P Shah at al observed sensitivity of 80% and specificity of 62% of haemoglobin colour scale method in comparison to Sahli method when haemoglobin cutoff level was kept 11 g/dl [10]. Tatsumi N et al in west Java, Indonesia, found Sahli and copper sulphate method had similar sensitivity and specificity [12]. Lewis SM and Emmanuel BJ [13] and Timan IS et al [14] also observed no differences between sensitivity and specificity of Sahli method, copper sulphate method and haemoglobin colour scale method.

Certain advantages and disadvantages were observed in all the three methods which are mentioned below.

### **Cyanmethaemoglobin method**

#### **Advantages**

- It is accurate, acceptable and standard.
- All forms of Haemoglobin, carboxyglobin & methemoglobin except sulphhemoglobin can be measured.

#### **Disadvantages**

- It is too expensive for the use at the primary health care level.
- Worker has to collect sample from the fields and bring it to PHC level (under non exposed condition).
- Test requires specific Drabkins solution which is poisonous.
- Moreover test requires colorimeter which requires electricity.
- It may not make available at specific time.
- Hb solution fades rapidly and must be read within 6-8 hrs.

### **Sahli's Method**

#### **Advantages**

- It gives satisfactory results
- If it is manufactured at large scale, it would be fairly cheap
- It can be provided easily at PHC level.

#### **Disadvantages**

- It requires same technical competence and enough training provided to worker
- It gives immense work load on field health worker making difficult to do Hb% estimation on each pregnant mothers while visiting in the field.
- There are chances of breakage while touring and sometimes non availability of standard quality of N/10 HCL.
- While sucking the blood into pipette there are chances of blockage, which require special stillate, again there is chances of air bubbles which can give rise false results.
- If one estimation is performed then one has to wait for the second estimation till the complete dryness of hemoglobin estimation glass tube otherwise false results will be obtained.

- Non hemoglobin substances (protein and lipid) in plasma and cell stroma in the test tube will alter the readings.
- All forms of hemoglobin can't be measured.

### **Copper sulphate method**

#### **Advantages**

- It gives quick, satisfactory results, very cheap easy to use and does not require technique.
- Copper sulphate solution can be made at PHC level and it may remain stable for longer time.
- It can screen large number of donors for possible anaemia.
- If provided in plastic container the chances of breakage can be ruled out while carrying into the field.
- Do not require any special technique and skill hence any lay person can do it with minimum training.
- Do not require any special reagent.
- Its sensitivity is too high as 96.55% while specificity 71.42% at 11gm% level.

#### **Disadvantages**

- This is not quantitative test.

## **Conclusion**

Result by different methods are variable and for this reason the International Committee for standardization in Hematology recommended in 1996 that the Cyanmethaemoglobin method should be exclusively used for determination of blood haemoglobin. This has now been accepted universally. Though the method is accurate it is not feasible at PHC level. The Sahli's method has sensitivity of 86.20% at 11gm% and hence Sahli's method can be used in ANC clinic and at primary health centre where all the facilities are made available. Copper sulphate has accuracy of 96.52% at 11gm% and can be used in field. Copper sulphate and Sahli's method should be exclusively provided at every primary health centre.

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