*ORIGINAL ARTICLE*

# Copper Sulphate Densitometry as a Screening Method for Anaemia in Pregnancy

**Wondossen Semie, BSc[1*], Wondossen Abate, BSc[2], Negussie Wodajo, MSc[2]**

## ABSTRACT

**Background:** *The prevalence of anemia in pregnant women reaches 65% in South Asia and 63% in Africa. The prevalence in Ethiopia in pregnant women ranges b/n 23.0%-66.5%. The need of having a simple screening method for anaemia is higher in a country like Ethiopia where the problem is serious, particularly in many of the rural areas. The objective of the study was to evaluate the accuracy, simplicity, and suitability in field use and cost effectiveness of the copper sulphate screening method for anaemia in pregnancy against the standard cyanmethemoglobin method.*

**Methods:** *Based on this fact a cross sectional study was conducted to evaluate the accuracy of copper sulphate screening method for anaemia in pregnancy against the standard cyanmethemoglobin method. In addition to this, the prevalence of anaemia in pregnancy was determined among 168 first-time attendants of antenatal care clinic, using the hemoglobin results obtained from the standard method, at Jimma Health Center, Jimma, Ethiopia from September 30 to December23, 1998.*

**Results:** *The two strengths of copper sulphate method (SP.G.1.044.and SP.G.1.049) proved to be simple and accurate in detecting a hemoglobin level <8g/dl and <11g/dl in pregnancy (sensitivity 95% and 96.2%, specificity 98.5% and 91%, positive predictive value 75% and 82.0%, negative predictive value 99.4% and 99%) respectively. The estimated cost of this screening method was six times less than the cost of the standard cyanmethaemoglobin method. The over all prevalence of anaemia was 31% the rate being 19.2% and 40.0% for urban and rural residents respectively. The majority (65.3%) had moderate anaemia, 28.9% mild anaemia and 5.8% had severe anemia.*

**Conclusion:** *In this study the copper sulphate method was found to be accurate, cheap, simple and can be recommended for screening for anaemia in pregnancy at primary health care level.*

**Key Words**: **Anaemia, copper sulfate, Hemoglobin, cyanmethemoglobin, Pregnancy.**

## INTRODUCTION

Anaemia has been extensively studied through out the world [1]. The over all prevalence of anaemia is about 30% world wide and 36% for developing countries. Among all groups, young children and pregnant women are most affected with an estimated global prevalence of 43% and 51% respectively. The prevalence of anemia in pregnant women reaches 65% in

[1] Ethiopian Health and Nutrition Research Institute, P. O. Box 5654, Addis Ababa, Ethiopia.
[2] Jimma university, School of Medical Laboratory Technology, P.O. Box 378, Jimma, Ethiopia.
*Corresponding author

South Asia and 63% in Africa (2, 3). In Ethiopia the prevalence of anemia in pregnant women ranges b/n 23.0%-66.5% based on few studies which have been conducted in some parts of the country (4-6).

Determination of haemoglobin (Hgb) or haematocrit (Hct) levels of pregnant mothers at least once in their anti-natal care (ANC) programme is useful for identifying serious degrees of anaemia for which intensive supervision and treatment schedules need to be arranged (7). Such effort is obviously essential if these pregnant mothers are screened in their own area and then treated locally or referred to other health care institution as required. However, in the developing countries even relatively simple laboratory techniques to do Hgb or Hct determination may not be available. This is particularly true in many of the rural areas where the prevalence of anaemia is most likely to be the highest and where appropriate prevention and treatment strategies may have the most beneficial outcome (8). Thus, early identification of anaeamic pregnant mothers, particularly those with severe anaemia, by a screening method at the rural health set up is more useful and improves the local ANC service.

Therefore, to screen anaemia in small laboratories with limited resources like in a health stations and health centers where there is frequently a lack of electricity and lack of laboratory personnel, simple, reliable and inexpensive screening method is required (9). In the absence of laboratory personnel other health workers assigned in these health posts play an important role in the fight against anemia. This is by doing simple screening methods other than the routine clinical and physical examination after a brief training on how to use and interpret those available methods (10).

Considering the drawbacks of using the clinical evaluation alone, several attempts have been made to evaluate the accuracy of other simple and reliable screening methods which can be used mainly for rural areas of the developing countries. Hence, copper sulphate method was the one that was studied in various areas. However, many of these studies were carried out using only one strength (cut-off point) to screen anaemia below 10.0gm/dl (11-13). Few interesting studies were done on copper sulphate by designing two strengths of the solution corresponding to 8gm/dL and 10gm/dL and also the study had shown the good participation of non-laboratory personnel like community health workers and clinic nurses in the screening procedures (10,14).

As to our knowledge no single study was conducted to evaluate the accuracy and the applicability of the copper sulphate screening method for anaemia in our country except the routine utilization of copper sulphate solution in some blood banking laboratories to screen blood donors for anaemia. The general principle of the the method is based on its standard solutions' capacity to measure the specific gravity of the whole blood or plasma by letting the drop to fall into a graded serious of copper sulphate solution to see whether the drop rise or fall in the solution (15).

In light of these, the primary aim of the present study was to evaluate the accuracy, simplicity, and suitability in field use and cost effectiveness of the copper sulpate screening method for anaemia in pregnancy against the standard cyanmethemoglobin method. Based on the Hgb results gathered from the standard method, a recent information on the prevalence of anaemia in pregnancy in Jimma area was also produced.

## MATERIALS AND METHODS

A cross sectional study was conducted to evaluate the accuracy of copper sulphate screening method for anaemia in pregnancy

against the standard cyanmethemoglobin method and the prevalence of anaemia was determined in the period between September 30 to December 23,1998. The study period and the sample size determination was based on the following assumptions and degree of confidence: 1. Previous prevalence of anaemia in the given study area. 2. The total number of pregnant mothers who visited the anti-natal care clinic of JHC for the first time in the previous year. c) Setting a confidence level of 95%. 3. The convenience of the study period to get adequate samples both anaemic and non-anaemic cases since various health professionals of Jimma Institute of Health Sciences. JIHS (Internship, B.Sc. Nursing and Medical laboratory Technology students) were assigned at Jimma Health Centre in the Team Training Program. Because this type of well-organized activity has an impact on strengthening the antenatal care activity in the given health institution. As a result of all these assumptions the sample size was calculated to be 180. However due to holy days and power interruption on working days. it was only possible to get a total of 168 first-time attendants during the study periods.

Therefore in this study all pregnant mothers whose residence was in Jimma town and its surrounding attending Jimma health centre antenatal care clinic for the first time in the present pregnancy were included. Those who had already visited the health centre before the study period were excluded. This was to avoid a falsely elevated result of Hgb that may happen as a result of early therapeutic or prophylactic iron and health education on nutrition at their first visit.

The willingness of each study subjects to participate in the study was asked and they were also informed about the questionnaire, laboratory investigation, and the individual and the social benefits of the study.

In the pre test, optimization of the two strengths of copper sulphate solutions was performed using non-anaemic blood samples, which were diluted with isotonic saline solution to obtain five samples with Hgb concentration of 6,7,10,10.8 and 12g/dL and their Hgb values were determined by coulter counter. Each sample was tested with the following five different strengths of copper sulphate solutions (SP.1.044, 1.046, 1.048, 1.049, 1.050). Based on the results observed in the pre-test, 1.044 and 1.049 were found to be the best strengths of solutions which corresponds to 8g/dL and 11g/dL of haemoglobin value. Therefore, these two strengths of the solution were prepared according to the protocol of Philips *et. al* (15). Using a hydrometer the strengths were checked after preparation and before daily use.

Anti-coagulated venous blood samples collected from the subjects were screened for anaemia by copper sulphate method using the two strengths (1.044 and 1.049) and also Hgb was determined by the standard method. The copper sulphate screening was performed according to Philips *et al* protocol. Hence, the copper sulfate at 11g/dL (SP.G.1.049) was used to screen for anemia and 8g/dL (SP.G.1.044) to identify those pregnant mothers with severe anemia. The cyanmethaemoglobin (HiCN) method was performed as recommended by the ICSH (16). Corning 252 colorimeter was used to read the absorbance and its linearity was also checked in the very beginning.

Other than the descriptive statistics, which was used for data analysis, the accuracy of copper sulphate method was calculated based on its sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) determination.

For the purpose of determining the presence of anaemia a Hgb cut-off level defined by WHO was used in this study (17).

## RESULTS

A total of 168 first-time attendants were identified and included in the study, the general characteristics of which are illustrated in Table 1. Anaemia (Hgb<11.0g/dL) was present in 52(31.0%) of the 168 study subjects. The mean age of the study group was 23.8 years with standard deviation of 5.5 and the youngest was 16 years old where as only 5 (3.0%) were above 35. The mean Hgb level for the whole group was 11.7g/dL and 8.6g/dL for anaemic women. The majority (65.3%) had moderate anaemia (Hgb7.0-10.0g/dL), 28.9% had mild anaemia (10.0<Hgb<11.0 g/dL) and 5.8% with severe anaemia (Hgb<7g/dL).

**Table 1.** General characteristics of pregnant women attending ANC for the first time at Jimma Health Center, September to December 1998.

| Variables | Anaemic (%) | Non-Anaemic (%) | Total (%) |
|---|---|---|---|
| Age | | | |
| < 20 | 19(28.4) | 48(71.6) | 67(39.9) |
| 21-25 | 15(31.2) | 33(68.8) | 48(28.6) |
| 26-30 | 10(29.4) | 24(70.6) | 34(20.2) |
| 31-35 | 5 (35.7) | 9 (64.3) | 14 (8.3) |
| >35 | 3 (60.0) | 2 (40.0) | 5 (2.9) |
| Residence | | | |
| Urban | 14(19.2) | 59(80.8) | 73(43.5) |
| Rural | 38(40.0) | 57(60.0) | 95(56.5) |
| Religion | | | |
| Muslim | 30(29.4) | 72(70.6) | 102(60.7) |
| Christian | 22(33.3) | 44(66.7) | 66 (39.3) |
| Ethnicity | | | |
| Oromo | 44(38.3) | 71(61.7) | 115(68.5) |
| Amhara | 0 (0.00) | 25(100.0) | 25 (14.9) |
| Gurage | 3 (30.0) | 7 (70.0) | 10 (5.9) |
| Others | 5 (27.8) | 13(72.2) | 18 (10.7) |
| Educational status | | | |
| Illiterate | 33(39.8) | 50(60.2) | 83(49.4) |
| Literate | 19(22.4) | 66(77.6) | 85(50.6) |
| Parity | | | |
| 0 | 8(15.4) | 44(84.6) | 52(31.0) |
| 1-4 | 20(27.0) | 54(73.0) | 74(44.0) |
| >5 | 24(57.1) | 18(42.9) | 42(25.0) |
| Gestational age(weeks) | | | |
| ≤13 | 4 (10.0) | 36(90.0) | 40(23.8) |
| 14-25 | 21(27.0) | 49(70.0) | 70(41.7) |
| >25 | 27(46.5) | 31(53.5) | 58(34.5) |
| Total | 52(31.0) | 116(69.0) | 168(100) |

Based on the study findings the prevalence of anaemia was higher (40.0%) in rural residents than urban (19.2%) (Table1). Likewise the prevalence of anaemia increased with gestational age of pregnancy where 24% first attended the ANC clinic during the first trimester, 41.7% in the second trimester and 34.5% in the third trimester in which the prevalence of anaemia was the highest (46.5%).

Figure 1 shows comparison of results obtained by the standard cyanmethaemoglobin and copper sulphate method for the detection of anaemia in three categories that were used in this study for technical purpose. Therefore, <8g/dL was used to identify those with severe anaemia where as 8-10.9g/dl for moderate or mild anaemia and ≥ 11g/dL was for non-anaemic pregnant women. Table 2 sets out the sensitivity, specificity, PPV and NPV at 8g/dL and 11g/dL of copper sulphate screening method. Likewise Table 3 shows the cost estimation to carry out a single test by the copper sulphate method in comparison to the standard method

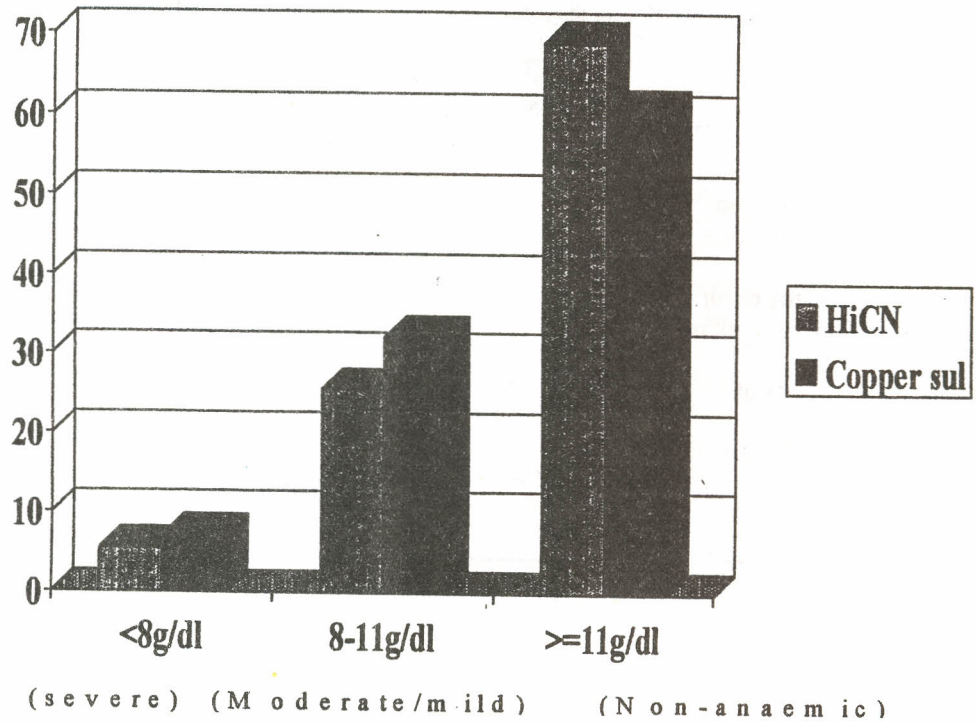


Fig 1. Comparison of HiCN and copper sulphate for the detection of anaemia, Jimma health center, 1998

**Table 2.** Sensitivity, specificity, PPV and NPV of the two strengths of copper sulphate solution, Jimma Health Center, September to December 1998.

| Strength of copper sulphate | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|
| Specific gravity.1.044 (at 8g/dL Hgb value) | 95.0 | 98.0 | 75.0 | 99.4 |
| Specific gravity.1.049 (at 11g/dL Hgb value) | 96.0 | 91.0 | 82.0 | 99.0 |

**Table 3.** Comparison between cyanmethaemoglobin and copper sulphate methods on different variables, Jimma Health Centre, September to December 1998.

| Variables | Cyanmethaemoglobin (HiCN) method | Copper sulphate method |
|---|---|---|
| Category of the test | Quantitative | Qualitative |
| Electrical requirement | Yes | No |
| Instrument requirement | Yes | No |
| Complexity of operation | High | Low |
| Level of skill | High | Medium |
| Time to obtain the result | 20 minutes | 2 minutes |
| Number of tests per 100ml solution | <20 tests | 50 tests |
| Approximate costs of 100 ml solution | 12 Ethiopian Birr | 5 Ethiopian Birr |
| Estimated cost per sample excluding the cost of electricity .man power. puncturing devices | 60 Ethiopian cents | 10 Ethiopian cents |

## DISCUSSION

The 31% prevalence of anaemia in this study is lower than that of Jimma and Assendabo where the prevalence were 41.9% and 66.5% respectively (5,6). One of the reason may be due to the smaller sample size obtained in this cross sectional study and the other could be due to the variation of the method where sahli's method was used in those previous studies. In comparison to the standard method used in this study, the sahli's technique was reported to be much less satisfactory under operational circumstances, chiefly because of dilution problems involved in the use of ordinary manual pipettes and subjective bias during visual comparison (18,19).
However, it is higher than the report from Gondar (23%) which may be due to the criteria to select the study subjects (4). Unlike Gondar report, in this study only first time attendants were included to avoid a falsely elevated haemoglobin estimation that might be obtained due to early prophylactic or therapeutic iron or folic acid treatment if all pregnant were included rather than selecting the first time attendants Though the primary aim of this study was to evaluate the copper sulphate method, it was possible to see also similar findings with previous reports, on the significant association ($p<0.01$) between anaemia in pregnancy and gestational age and the high prevalence of anaemia in the rural residents than urban residents (5, 6). The increasing demand of nutrients in relation to gestational age and the low standards of living, the poor family planning practice and the farm work with bare feet that exposes pregnant mothers to parasitic infection like hook worm infection at the rural place may be the possible reasons.
The Hgb values categorized by the two strengths of copper sulphate method showed 102 pregnant women (60.7%) had Hgb level > 11g/dL, 54 (32.1%) between 8-10.9g/dL and 12 (7.2%)<8g/dL (Fig.5). However, 16 cases (9.5%) were in a disagreement with the standard cyanmethmoglobin method in which 14 women (8.3%) had Hb under estimated (false positives) by the copper sulphate method. Of these 14 cases, 3 (1.8%) were categorized as severely anaemic while they were moderately anaemic (at 1.044 specific gravity) and 11 (6.5%) categorized as anaemic while they were not (at 1.049 specific gravity). The remaining two cases (1.2%) had Hgb levels over estimated (false negatives) by copper sulphate at 1.049 specific gravity. In practical terms, 90.5% of these pregnant mothers were immediately screened correctly for anaemia by the screening method in relation to the standard method.

The diagnostic sensitivity, specificity and negative predictive value of copper sulphate method in this study were similar in comparison with previous reports (10-13). However, the 100% sensitivity and specificity of the copper sulphate reported from Jamaica (10) were not found in this study. Nevertheless, the study findings for positive predictive value showed a better result for copper sulphate designed at 11 g/dl than many of those previously done at 10g/dL cut off point. But for the other strength designed at 8g/dL, more or less similar findings observed in comparison with those few studies in which the same strength of copper sulphate was included. The higher positive predictive value obtained in this study may be due to the newly designed and used cut-off point at 11g/dL with a specific gravity of 1.049.

According to the WHO, anaemia in pregnancy is said to exist when the Hgb level is below 11 g/dL at the first trimester and below 10.5g/dL in the second trimester as a result of a fall of 0.5g/dL due to the increase in plasma volume (2). In this study the above definition was employed to

identify pregnant mothers with mild anaemia particularly those in the first trimester with Hgb level between 10g/dL to 10.9g/dL and those in the second or third trimester with Hgb level between 10g/dL to 10.4g/dL. If this study was conducted using 10g/dL cut-off, some of the mild anaemic cases in the first trimester could have been missed. For instance, in this study 28.9% of the study subjects were with mild anaemia in which 26.7% of this were in the first trimester, 33.3% in the second trimester and 40.0% in the third trimester.

Since the purpose of screening is for the early detection of disease, it is better to have a cut-off point at 11g/dL of copper sulphate screening method. Possible limitations of copper sulphate screening method observed in this study are the technical difficulty of adjusting the specific gravity to the desired strength and lack of getting the specific hemoglobin result. However the latter disadvantage of the method is irrelevant for screening purposes. Therefore once the initial technical difficulties to prepare the given standard solution is solved, the solution is stable and no errors would result from deterioration as long as the stock solution is kept in a closed glass or plastic containers.

As it is shown in Table 3 the comparison b/n the copper sulphate and the standard cyanmethaemoglobin method was made on the estimated cost per test and also on other variables. Hence, excluding the cost of electricity, puncturing devices and man power, the estimated cost of screening pregnant mothers with copper sulphate solution is about six times less than (10 Ethiopian cents per sample) the cost estimate of Hgb measurement by standard method (60 Ethiopian cents per sample). Furthermore, the method is convenient, simple and rapid requiring two minutes per sample.

*Field Testing*: To assess the applicability of copper sulphate screening method a preliminary field trial was carried out at the Eladale peasant association which is found under the Jimma Health Center catchment area. Five health assistants were selected from Jimma hospital and Jimma Health Center. These participants went to the field site after theoretical and practical sessions were given for a maximum of two hours. They were able to screen a total of 57 women (45 lactating and 12 pregnant women for anaemia by copper sulphate method and at the same time haemoglobin was determined by HiCN method for all of these women by one of the investigators. Only 6(10.5%) women (four pregnant) and (two lactating) were found to be anaemic (9.0-10.8g/dL) by HiCN method. Including these six, other four samples with 11.0g/dL to 11.2g/dL Hgb levels (false positives) were identified as anaemic by the screening method.

Actually very few technical difficulties were encountered by some of the health assistants due to lack of sufficient training and the smaller number of anaemic subjects found by the preliminary field trial. Otherwise it is promising that this screening method is to be used in areas where scarcity of laboratory personnel and facilities are present. However due to financial constraints extensive field trial was not carried out in this study. Therefore, the investigators would like to suggest a further investigation to be carried out on the field trial.

In conclusion, our aim was to find out, how reliable is copper sulphate screening method to detect anaemia. Based on this fact, the simplicity, cost effectiveness, applicability in the rural areas where lack of laboratory professional, electricity and technologies exists makes the copper sulphate screening method very useful to control anaemia. Though some discrepancies and problems were observed in comparison with the standard method.

copper sulphate screening method has its own advantage to be used as a screening method particularly in the rural areas where the prevalence of anaemia is higher and in a country where appropriate technology is required. Due to the technical difficulties which occur during the preparation and determination of the specific gravity of the standard stock solution of copper sulphate, the investigators would like to suggest this stock solution to be made at a central or regional point and distributed every few months to health stations or health centers. Furthermore the very limited field trial conducted in this study deserves a further extensive field investigation with appropriate training of non-laboratory personnel.

## ACKNOWLEDGEMENTS

This study was financially supported by the Research and Publications Office of the then Jimma Institute of Health Sciences. Ethiopian Health and Nutrition Research Institute is also gratefully acknowledged for material provision and the scholarship given to the first author.

## REFERENCES

1. Masawe AEJ. Nutritional anaemia in tropical Africa. In: Luzzatto L (ed.) Clinics in Haematology. Butler & Tanner Ltd, London 1981, p 5.
2. WHO. Report of a technical working group on prevention and management of severe anaemia in pregnancy. WHO. Geneva. 20-22 may 1991. p.10.
3. Demaeyer E. Adiels-Tegman M. The prevalence of anaemia in the world. WHO Quarterly Rep 1985; 38:302-316.
4. Peter WH. Hematocrit & haemoglobin levels in adult males and in pregnant and non-pregnant females in Northern Ethiopia. *Ethiop Med J* 1984; **22**:17-26.
5. Solomon D. Prevalence of anaemia in pregnancy in Jimma town, South western Ethiopia. *Ethiop Med J* 1993; **31**:251-258.
6. Yonas, T. Prevalence of anaemia among ANC attendants of Assendabo Teaching Health Center (dissertation). Jimma Institute of Health Sciences. Jimma, 1996.
7. Pappagollo S, Bull DL. Operational problems of an iron supplementation program for pregnant women an assessment of UNRWA experiences. *Bull Wld Health Org* 1996; 74:25-32.
8. Axemo P, Bregstrom S, Kollstedt E, Lindmark G, Molin A, George W, *et al* (ed). International maternal health care. WHO, 1995; pp.47-50.
9. Johns WL, Lewis SM. Primary health screening by haemoglobinometry in tropical community. *Bull WHO*, 1989; 67:627-633.
10. Topley E. Anaemia in rural Africa: Community support for control activities where malaria is common. FSG Medimedia Ltd.. London 1998. p 4.
11. Wilkinson D, Sach ME. Cost effectiveness on site screening for anaemia in pregnancy in primary care clinics. *South Afr Med J* 1997; 87: 463-465.
12. Pistorius LR, Funk M, Pattinson RC, Howarth GR. Screening for anaemia in pregnancy with copper sulphate densitometry. *Int J Gynacol Obstet* 1996; 32 (1): 33-36.
13. Lewis SM, Stott GJ, Wynn K.J. An expensive and reliable haemoglobin colour scale for assessing anaemia. *J Clin Pathol* 1998; 51:21-24.
14. Shah U, Pratinidhi AK, Bhatta PV, Wande, Alekar SV. Anaemia screening by community health workers. *World Health Forum* 1984; 5:35-38.

15. Philips RA, Van Slyke DD, Hamilton PB, Dole VP, Emerson K, Archbold RM. Measurement of specific gravities for whole blood and plasma by standard copper sulphate solutions. *J Biol Chem* 1950; 183: 305-330.
16. International Committee for Standardization in Haematology. Recommendations for reference method for haemoglobinometry in human blood and specification for international haemoglobincyanide reference preparation. *J Clin Pathol* 1978; 31:139-142.
17. WHO. Iron Deficiency: Indicators for assessment and strategies for prevention. WHO/NUT/96.12.WHO, Geneva.
18. Stone JE, Simmons WK, Justum PJ, Gurney JM. An evaluation of methods of screening for anaemia. *Bull Wld Health Org* 1984; 62(1): 115-120.
19. Leerberghe V, Keegels W, Cornelis G, Ancona C, Mangel Schots E, Vanbalen M. Haemoglobin measurement: the reliability of some sample techniques for use in primary health care setting. *Bull Wld Health Org.* 1993; 61 (6): 957-965.