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A Multi Method Assessment of Anaemia and Response to Iron Supplementation among Women in West Bengal, India

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Anaemia is a broad term applied to the condition in which there is inadequate or defective formation of haemoglobin, or defective maturation and formation of red blood cells[1]. The term "anaemia" is sometimes used synonymously with "iron deficiency anaemia"[2]. There are also many causes of anaemia besides iron deficiency, particularly in tropical regions. In any case, however, iron deficiency is the predominant nutritional deficiency causing anaemia and is present even when other causes of anaemia are recognized.

Iron deficiency affects a significant part of the population in nearly every country in the world[2,3]. Iron deficiency anaemia is a major public health problem in developing countries like India[4,5]. The prevalence of iron deficiency varies greatly according to host factors: age, gender, physiological, pathological, environmental and socio-economic conditions[6].

It is evident from National Family Health Surveys (1998-99 and 2005-06)[7,8] that anaemia is widespread among women in reproductive ages. According to NFHS-3, overall fifty five percent of women have some degree of anaemia which is in reality a very large public health concern. Prevalence of anaemia is particularly high in eastern region of India consisting of states of Bihar, Jharkhand, Orissa and West Bengal. It is a matter of concern that in these areas prevalence of anaemia is clustering, and this perhaps may be an area specific problem. Clustering of cases in this region suggests that this deficiency is not related to individual health problems but has to do with the availability and adequacy of food rich in nutrition in this region. Therefore there is an urgent need to examine whether such a high prevalence of anaemia is due to iron deficiency or any other reason. A community level study in the area where the level of anaemia is higher will be helpful from the policy and programme perspective[9].

The main goal of this paper is to evaluate the usefulness of symptoms, four clinic indicators and low cost method of hemoglobin concentration test for primary care where resources are scare and anemia is wide spread. The other objective of this study is to examine iron deficiency anemia among the women who were suffering from any anemia.

Methods

Sampling

As the prevalence of anaemia was found universal in most of the districts of West Bengal as per NFHS-II, only five districts which were in proximity with each other had been purposively selected for logistic convenience. Anaemia level was examined for all the Primary Sampling Units (PSUs) of these five districts of West Bengal sample of National Family Health Survey – II[7]. From each selected district, one high prevalence (> 40 percent women in reproductive ages suffering from any degree of anaemia) and low prevalence (≤ 40 percent women in reproductive

ages suffering from any degree of anaemia) village were selected. From each selected village, 50 households were selected by systematic sampling. From each selected household, all the women in the reproductive ages (15-49) were interviewed. A total of 529 households were covered and a total of 559 ever married women in the age-group of 15-49 were interviewed[10].

Assessment of anaemia

The choice of diagnostic methods for anaemia depends on the purpose of the assessment and the resources available[11]. In this study an attempt is made to show how useful are symptoms and clinical indicators in identifying sever and moderate anaemia (below 10 g/dl) cases in rural setting of India (See Figure 1). The other goal is to examine usefulness of simple haemoglobin concentration method in identifying any anaemia (below 11 g/dl for pregnant and 12g/dl for non-pregnant women).

Symptoms

Self reported symptoms are considered as first step in diagnosis of severe and moderate anaemia. In this study symptoms namely weakness, dizziness, breathlessness, sores in the mouth, weight loss, frequent headaches, gastric problems etc., are collected.

Clinical indicators

Clinical indicators (visual inspection of physical characteristics) are non-invasive procedures for identifying anemia. In these methods of screening, only investigators required sufficient training to identify the anaemic cases [12,13,14,15,16]. This procedure is less costly and readily accepted by the participants, as it will not require blood sample. As well as it is useful in setting with minimal laboratory facilities, expendable goods are in short supply and health care providers have only basic minimum training[11]. The use of clinic indicators is recommended for screening for treatment of cases with severe anaemia. Management of severe anaemia may provide one strategy to reduce the need for transfusion of blood under the prevailing risk of human immunodeficiency virus (HIV) transmission via blood transfusion in resource poor countries[16,2].

Laboratory tests

The prevalence of anaemia in a population is best determined by using a reliable method of measuring haemoglobin concentration[17]. The determination of the prevalence of anaemia in a population is relatively simple and inexpensive by most of the methods. The low cost and simplicity of operation of a screening test is useful at community level in rural areas. A simple method of haemoglobin test is Helliges method where colour of blood is matched with the colour chart and readings of haemoglobin concentration are noted. As a gold standard method, Cyanmethemoglobin (CYAN-MATH) method is used to examine sensitivity and specificity of clinical signs and low cost method. The Cyanmethemoglobin method for determining haemoglobin concentration is the best laboratory method for the quantitative determination of haemoglobin. This method is the international standard for hemoglobin determination, as stable reference solutions are available for calibration[11]. It serves as a reference for comparison and standardization of other methods[17]. In this method, a fixed quantity of blood is diluted with a reagent (Drabkins solution) and haemoglobin concentration is determined after a fixed time interval in an accurate, well-calibrated photometer.

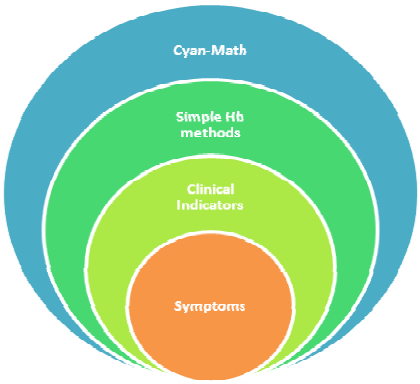

Figure 1: Different tests for anaemia

Intervention

Iron status can be determined by several well-established tests in addition to measurement of haemoglobin[18,11,2]. The use of multiple tests only partially overcomes the limitation of a single test[19]. The use of multiple tests is not an option in resource-poor settings because of their relatively high cost. Moreover, iron-related tests do not all correlate closely with one another because each reflects a different aspect of iron metabolism[20]. Hence established approach to the diagnosis of iron deficiency in individuals by monitoring changes in haemoglobin after oral iron supplementation is adopted in this study[21,22].

Field procedures

In order to upgrade the skills of investigators who were primarily taken from a local NGO working in the area of health, comprehensive training was organized. Training was imparted by the principal investigators regarding interview technique, measurement of physical work load, and quantity of food items taken by the respondent by using measuring jars, glasses, and cups[23]. Special lecture was given on the symptoms of anaemia and related morbidity.

In order to train investigators about clinical indicators, special session by medical doctors have been arranged, as well as they had undergone internship in the hospital before going to field. Investigators are trained to identify four clinical indicators namely pallor of the eyelids, palms, nail beds and tongue. All clinical signs are categorized into three categories namely normal, pale and very pale. Clinical indicators are used in this study to identify the moderate and severe anaemia cases as suggested by WHO[2]. Indian women apply *'Hina'* (tattoos) on palm on marriage or any other celebrations as well as nail colouring is also common. In some cases women have used turmeric on their hands. Apart from hand and nail colouring, some women were found eating betel leaves. Conjunctivae readings are not taken for women who were suffering with conjunctivitis, as it can cause redness even in anaemic subjects. Hence, in some cases one or two clinical indicators were not readable[24]. All women with or without symptoms or clinical indicators were called for blood test.

Consent of participants is taken before asking them to undergo blood test. Testing of haemoglobin concentration is carried out by the trained pathologists from reputed laboratory. Pathologists are called on the last day of data collection to the villages. Laboratory has supplied all the equipment and materials for testing haemoglobin. A mini laboratory is made in the convenient place in the village with water and electricity facility. Blinding of clinical indicators is done by not revealing the result to pathologists. One pathologist carried out visual colour match method and another CYAN-METH method, and recording of concentration is done by

investigators. Hence blinding of two laboratory methods for haemoglobin concentration is ensured. Concentration of haemoglobin is recorded on three copies, one is given to participants; other is attached with questionnaire and third is shown to medical doctor to prescribe iron supplement to the anaemic women. Iron supplementation (60 mg iron and 400 µg Folic Acid) is given by the local primary health care provider. One iron tablet daily is recommended as it will increase consistent use and it is preferable to reduce the risk of total rejection or non-acceptance of supplements[14,25,2]. All anaemic women were well informed about the health benefits - as well as the side-effects - of iron supplementation. After one month of iron supplementation to women who were anaemic, again blood test by CYAN-METH method is carried out. Those women who have not shown any improvement have been advised to take tablets for two more months.

Blood test is carried out on the last day of survey in the village at one place convenient to establish mini laboratory. Some women who were going for work and did not find time and place convenient for the blood test have not participated in the study. In general, when camp was held within two days of interview of women, turn over was high. Four hundred and forty four women in the the baseline survey and 287 women in the follow-up have undergone the blood test.

Statistical analysis

The validity of symptoms and clinical indicators is tested against gold standard method. Clinical indicator is divided in normal, pale and very pale. As concentration of haemoglobin is not estimated using clinical indicators, hence it is tested against moderate/ severe and normal/mild as categorical variable instead of interval scale values of haemoglobin concentration of gold standard test. Screening test accuracy is frequently characterized by two simple measures, the sensitivity and specificity, reflecting two types of error in diagnosis. Sensitivity and specificity of all symptoms are examined by its presence and absence against severe/moderate and mild/normal haemoglobin conditions. Sensitivity and specificity of all clinical indicators are examined separately taking pale and very pale as well as combing together.

Receiver operating characteristic (ROC) analysis is based on classic model of signal detection theor[26,27,28]. This signal detection experiment was used in the evaluation of a diagnostic test that discriminates diseased from normal cases or subjects in epidemiological research[29,30]. Before describing results, let us define some notation. The true disease status for the case being tested is a known binary random variable, denoted by D, with D = 1 if the case is diseased and 0 if it not (Total cases 'n' consist of n_1 non-diseased and n_2 diseased cases). Let X and Y are independent random variables on the diagnostic test of groups of non- diseased and diseased cases. Two probabilities, specificity {Pr(screening test negative $\mid$ D = 0) = Pr(X $\geq$C) for a diagnostic criterion C} and sensitivity{Pr(screening test positive $\mid$ D =1) =Pr(Y < C). A graph plotted with these two probabilities {1-Pr(X $\geq$C) and Pr(Y< C)} for all values of 'C' is termed the ROC curve. A ROC curve is a plot of the true positive ratio, the sensitivity, against the false positive ratio, the specificity subtracted from one. Area under this curve is estimated using Wilcoxon -Mann-Whitney (WMW) statistics[31]. A ROC curve is usually chosen as the summary measure of diagnostic accuracy[26].

Use of ROC curves is widely accepted in evaluating screening tests of radiology also in psychiatric screening test[32,33]. In this paper an attempt is made to examine effectiveness of screening tests of anaemia against gold standard (CYAN-METH) using ROC curve. Clinical indicators are adjudged on ordinal scale; hence, an attempt is also made to study the ROC area for clinical indicators too. Using clinical indicators anaemia is predicted through logistic analysis for those women who have not participated for blood test.

To examine changes in the haemoglobin concentration after iron supplementation, frequency distribution is used. ANOVA and Post hoc method for multiple paired comparisons is also used to examine changes before and after iron supplementation[34]. Apart from these analyses 'two step cluster analysis' is also used to identify the group of women who have not responded to iron supplementation. The two step cluster analysis procedure is an exploratory tool designed to reveal natural groupings (or clusters) within a data set. This procedure creates clusters based on both categorical and continuous variables. This analysis uses a likelihood distance measure which assumes that variables in the cluster model are independent[35].

Sample characteristics

Women's health seeking behavior is invariably linked with their characteristics such as age, education, marital status, religion and caste. Forty percent women are concentrated in the age group of 15-29 years. The respondents comprised of mainly currently married women (96 percent). Nearly 45 percent of the women are illiterate. Sixty-three percent of the women are Hindus and 37 percent are Muslims. About 60 percent of the women were married below 18 years.

Water sources and sanitary facilities have an important influence on the health of the household members. In the survey it was found that 87 percent of the households use hand pump/bore well as the major source of drinking water, 11 percent use public tap for drinking water, and less than two percent use private tap as a source of drinking water. Regarding sanitation facilities 37 percent households have own toilet with flush facility, 26 percent use pit toilets, 3 percent use shared public toilet with flush facility and another 4 percent use public pit toilet that is shared whereas 31 percent have no toilet facility at all. Standard of living is another important measure of socioeconomic status of the household. In the surveyed villages twenty nine percent of the households have a low standard of living, 53 percent households have a medium standard of living, and 18 percent have a high standard of living[10].

Results

Three levels of severity of anaemia have been identified mild anaemia (10.0 – 10.9 g/dl for pregnant women and 10.0 – 11.9 g/dl for non pregnant women), moderate anaemia (7.0 – 9.9 g/dl), and severe anaemia (less than 7.0 g/dl)[36]. Ninety four percent of women have any anaemia in the study sample. A total of 49 percent women are moderately anaemic, and 45 percent are mildly anaemic and less than one percent women are severely anaemic.

Screening tests

The self reported symptoms of anaemia showed discriminatory ability when cases are classified as presence/ absence of symptom with respect to haemoglobin concentration by pathological test CYAN –MATH categorized as "normal- mild/moderate -severe". Only dizziness, weakness and gastric problems have shown more than 20 percent sensitivity (Table 1). For these three symptoms, specificity ranges from 70-80 percent. All other symptoms showed low sensitivity but very high specificity. On the whole, accuracy of symptoms is not very good in identification of cases for anaemia.

Only four female investigators who have received training in the hospital have performed assessment of clinical signs. Perception of colour does not vary among the investigators. Results are similar in the first and second half of the study. Hence intra and over period difference are negligible in reading of clinical indicators; it is useful to examine accuracy of these indicators against gold standard.

As a first step of examining clinical indicators, they are classified into two categories as any pallor and normal and tested against haemoglobin concentration by pathological test CYAN –MATH with cutoff for classification of anemia as 9.9g/dl. The four clinical indicators show higher level of sensitivity in comparison to all the symptoms collected in this study. Sensitivity ranges from 53 percent for pallor of palm to 91 percent for pallor of eyelids. Specificity for palm, tongue and nail is ranging between 80-65, whereas for eyelid it is 29 percent. To evaluate clinical indicators reading are ordinal or not, box plots (which uses median and quartile deviations) of haemoglobin concentration for these three categories namely normal, pale and very pale for all clinical indicators are examined. It is interesting to note that under normal condition of clinical indicators, haemoglobin is higher than pale or very pale conditions (Figure 2). Similarly, for pale condition, haemoglobin concentration is higher than very pale category. Hence, we can use these reading as observations on ordinal scale.

Figure 2 here

As these indicators are rated on ordinal scale, it is possible to examine these indicators' usefulness using ROC analyses. ROC area and standard deviation for each clinical indicator are given in Table 1. The areas under the curves are similar for four clinical indicators, although the colour of eye appears to have better than other indicators. The differences in the area are insignificant and hence any of the indicators is good enough for identification of anaemia with 0.65 accuracy level (Figure 3).

Table 1 and Figure 3 here

Accuracy of visual colour match method is first examined by considering the cutoff of 9.9 g/dl haemoglobin concentration against gold standard cutoff of 9.9 g/dl concentration. Sensitivity and specificity of this method is found as 84.5 and 90.4 percent respectively. Although sensitivity and specificity is very high but this comparison involves only one cut off point. Since the ROC cures involves all possible classification points, the calibration of these points ingrained in ROC analysis. In case of screening blood test accuracy of any anaemia (cutoff 12 g/dl) is 0.95 according to the area of ROC curve (Figure 3). In both the methods, haemoglobin concentration is recorded on interval scale; therefore, Pearson correlation, and paired T test comparison is also made. Pearson correlation between these two methods is 0.889 and mean deviation is 0.07324 with 95 percent confidence interval of 0.0229 to 0.1235. Such a high level of accuracy is achieved may be due to the fact that pathologist had performed screening blood test. In case of less experienced person also this accuracy can be achieved with little training. This is best substitute for CYAN –MATH method that required equipment, skills and cost is high.

Prediction of anaemia is carried out for those women whose blood examination reports were not available due to several non response reasons. Logistic analysis is carried out using clinical signs to predict the missing values. Only three clinical signs namely palms, tongue and eyes were used for logistics analysis as nails and palms are highly correlated[10]. Result of logistic analysis is presented in Table 2. Odd of having severe or moderate anaemia is significantly higher for those women whose clinical signs are positive (any pallor). Further prediction based on probability group from logistic analysis shows the distribution of predicted prevalence is similar to the observed level.

Table 2 here

Response to iron supplementation

Based on the clinical history and haemoglobin concentration level in the base line survey, doctors have prescribed iron supplementation to women in reproductive ages. There were 352 women who were found anemic based on the cutoff of 12 g/dl. Community level health workers have given iron supplementation for its correction among women for one month. At the end of one month, mini laboratory was arranged in the village. Second camp was held in most of villages in the summer. As in summer season mobility among women is high due to school holidays of children and around 48 women were out of station. Another 13 women refused and other four had not taken tablets at all. A total of 287 women participated in the second round for monitoring changes of haemoglobin concentration.

Percentage distribution of cases by haemoglobin concentration before and after iron supplementation is shown in the Figure 4 A and median and quartile deviations of follow-up blood test against level of anaemia before supplementation is shown in Figure 4B. From figures it is very clear that the haemoglobin concentration have improved significantly after iron supplementation. A further analysis of variance (ANOVA) is carried out to examine mean improvement in haemoglobin concentration by the level of anaemia women had before iron supplementation. Mean improvement in haemoglobin concentration by level of anaemia women were experiencing before iron supplementation is significantly different. In case of severely anaemic women a 3 g/dl improvement is found although number of women was less in this category followed by moderate and mild anaemic women. ANOVA results showed that differences exist among the means, post hoc range tests and pair-wise multiple comparisons can determine which means differ; hence an analysis is carried out for post hoc multiple comparison. From Table 3 it is clear that all pairs have significantly different means. Hence, it may be said that iron supplementation will not lead to uniform improvement among women.

Figure 4 here

A paired comparison of reading of two tests is also done and from this it is found that 63 percent women have shown improvement another 2.6 percent are at the same level. Other studies had reported that an increase of at least 1 g/l in haemoglobin after 1 or 2 months of supplementation is indicative of iron deficiency[37,2]. In the present sample, 16 percent women have shown one or more g/dl improvement in haemoglobin concentration. However, according to WHO[2], in areas where the prevalence of anaemia among women of childbearing age is severe (> 40%), preventive/corrective iron supplementation of 60 mg/day iron with 400 μg folic acid for 3 months should be considered.

Table 3 and 4 here

An attempt is made to identify the characteristic of those women who have not shown improvement in haemoglobin level after iron supplementation using two stage cluster analysis (Table 4). Only two variables namely weight and height is used for this analysis. It is quite interesting to notice that those women whose haemoglobin concentration is not increased belong to category of lower weight and shorter height. This shows chronic nutritional deficiency among women. There is also possibility that these women must be suffering from other form of anaemia or diseases too. In our sample two women have reported that they were suffering from thalassaemia.

Conclusions

The goal of this study was to evaluate using receiver operating characteristic (ROC) analyses, the ability of four clinical indicators that identify anaemia condition at community level. The participants in this study are women in reproductive ages from West Bengal, a state located in eastern part of India. ROC analysis showed that all four clinical indicators were able to discriminate between those with and without severe and moderate anaemia. Discriminatory ability did not differ significantly among the four clinical indicators, although the areas under the ROC curve were slightly higher for the pallor of eyes. The similarity of discriminatory ability for anaemia through clinical signs suggests its possible usefulness in other settings as well as their clinical value as screening tests. The major findings emerged from this study is that clinical indicators option is certainly better than symptoms in identification of severe and moderate anaemia. Hence health workers in the villages may be trained to identify the anaemic women, children and men.

Screening test of haemoglobin concentration with visual match also showed very high level of accuracy by ROC. This method may be preferred when compared to costly approaches at community level as well as in prevalence surveys of anaemia. HemoCue which is comparable to CYAN-MATH procedure is very costly method and it is not possible for small grant project to use this system and its recurring cost[11]. It may also be concluded that ROC analysis is useful for comparing anaemia screening test.

Looking at the response to iron supplementation in the study it may be concluded that majority of the women were suffering from iron deficiency anaemia. Improvement in haemoglobin concentration is not uniform among women who were at different level of anaemia before iron supplementation. All those women who have not shown improvement in haemoglobin concentration were with low weight and short height which is chronic nutrition deficiency. This finding required further research to examine why these women have not responded to iron and whether they are suffering from any other form of anaemia.

Although reliability of methods used here has been tested, there are inevitably limitations that resulted from non-response rate of anaemia tests. This could have been improved if we would had laboratory facilities and pathologist for all days during field work. Additionally, use of colour charts may enhance the capability of investigators for identification of pallor of eye, tongue, palm and nails. Thirdly, we have not collected changes in food intake by women after knowing the result of first test. Constant supervision and monitoring for intake of iron supplement is required at least on weekly basis.

The Government of India launched National Rural Health Mission (NRHM) for providing integrated comprehensive primary health care services, especially to the poor and vulnerable sections of the society[38]. One of the key components of the NRHM is to provide every village in the country with a trained female community health activist – 'ASHA' or Accredited Social Health Activist. AHSA is selected from the village itself and trained to work as an interface between the community and the public health system. Capacity building of ASHA is being seen as a continuous process. She will have to undergo series of trainings to acquire the necessary knowledge, skills for delivering health care facilities. Hence, ASHA may be trained in identifying the clinical sings of anaemia and this should be regularly used to screen individual women to identify high-risk cases before the onset of life threatening complications. This is likely to result in less demand of blood and avoid threat of HIV by blood transfusion.

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Table 1: Accuracy and Screening Tests

Screening Tests	ROC[1]		Sensitivity(%)[2]	Specificity (%)[2]	Number of women
	Area	Standard error			
Simple Haemoglobin test					
Helliges method	0.9535	0.0215	84.5	90.4	409
Clinical Indicators					
Pallor of eyelid	0.6962	0.0262	90.8	28.8	442
Pallor of tongue	0.6479	0.0278	58.1	71.9	436
Pallor of palm	0.6648	0.0275	52.5	80.2	395
Pallor of nail	0.6466	0.0278	60.3	65.9	442
Symptoms					
Weakness	NA[3]	NA	28.3	75.0	444
Dizziness	NA	NA	28.8	70.1	444
Gastric problem	NA	NA	23.7	79.9	444
Frequent headache	NA	NA	18.7	80.8	444
Sores in the corner of the mouth	NA	NA	8.7	95.1	444
Weight loss	NA	NA	8.2	96.9	444

Breathlessness	NA	NA	3.2	96.0	444

1. Response Operating Characteristic (ROC) curve for visual colour match is based on cutoff of 12 g/dl and for all clinical indicators cutoff for ROC cures is 10 g/dl for gold standard test.
2. Sensitivity and specificity for all screening tests is based on cutoff of 10 g/dl for gold standard test.
3. NA: All symptoms are based on binary scale with information on presence or absence of symptoms. Hence ROC analysis is not possible (NA –Not applicable).

Table 2: Results of Logistic Analysis

Clinical signs	**B**	**Wald**	**Significance**	**Exp(B)**
Palms	1.080	19.629	0.000	2.944
Tongue	0.690	8.060	0.005	1.993
Eyelids	0.933	9.411	0.002	2.542
Constant	-1.421	28.09	0.000	0.241
Prediction of anaemia based on logistic analysis		**Normal/Mild**	**Moderate/ Severe**	**Number**
Sample		50.5	49.5	444
Prediction		51.5	48.6	500
Dependent variable: 1-moderate to severe anaemia, 0- mild to normal Independent variables: 1- any pallor, 0 – no pallor Nagekerke R Square - 21.1 percent				

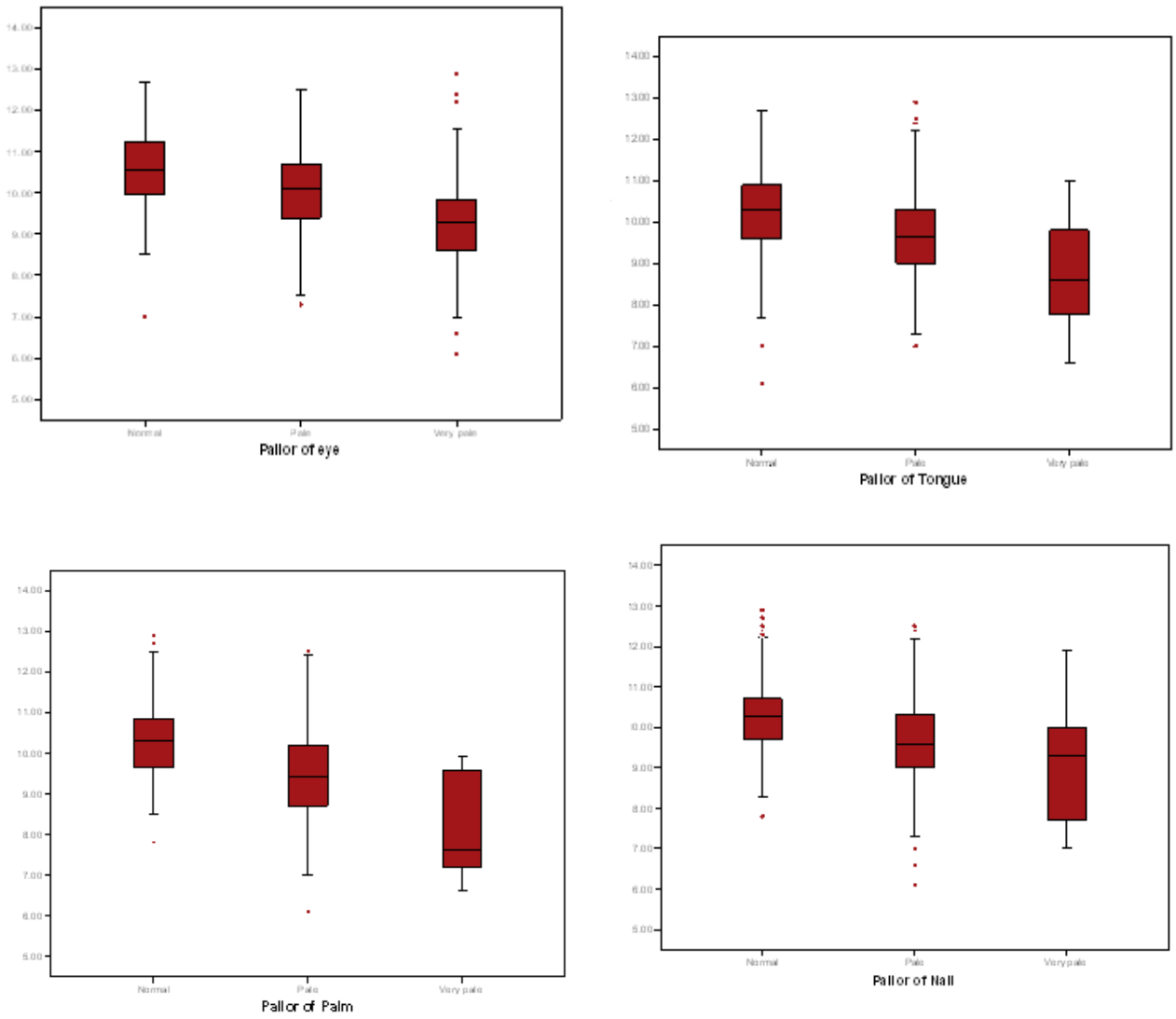
Table 3: Multiple comparison of haemoglobin improvement with level of anaemia before iron supplementation

Anaemia level		Mean Difference	Std. Error	Sign.	95% Confidence Interval	
I (category)	J (category)				Lower	Upper
Severe	Moderate	2.4462	0.5616	0.00	1.123	3.7693
	Mild	3.1796	0.5623	0.00	1.8548	4.5044
Moderate	Severe	-2.4462	0.5616	0.00	-3.769	-1.1230
	Mild	0.7335	0.0940	0.00	0.5119	0.9550
Mild	Severe	-3.1796	0.5623	0.00	-4.504	-1.8548
	Moderate	-0.7335	0.0940	0.00	-0.955	-0.5119

Table 4: Identification of group which have not responded positively by two step cluster analysis

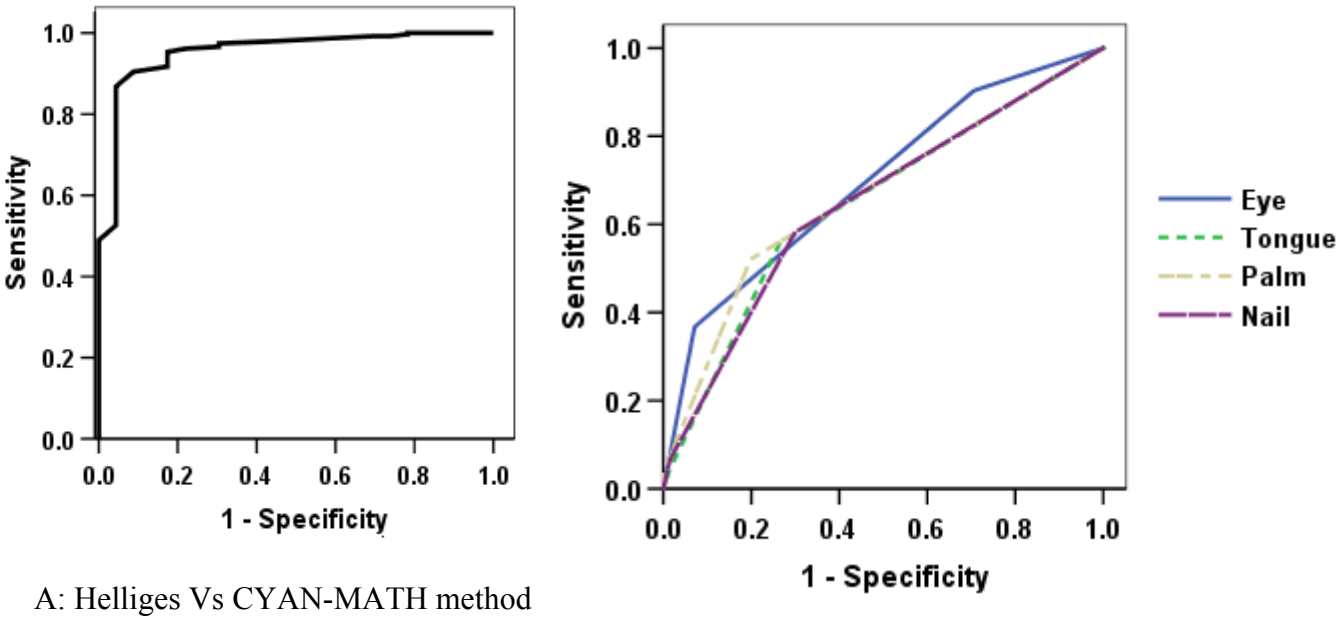
Characteristics	**Cluster1**	**Cluster2**	**Total**
Haemoglobin after iron supplementation			

Improved (No.)	187	-	187
Not improved (No.)	-	99	99
Weight(Kg)			
Mean	46.4433	44.3939	45.7339
Standard deviation	7.8183	9.2693	8.3899
Height(Mt)			
Mean	1.5149	1.4893	1.5060
Standard deviation	0.0543	0.1608	0.1047



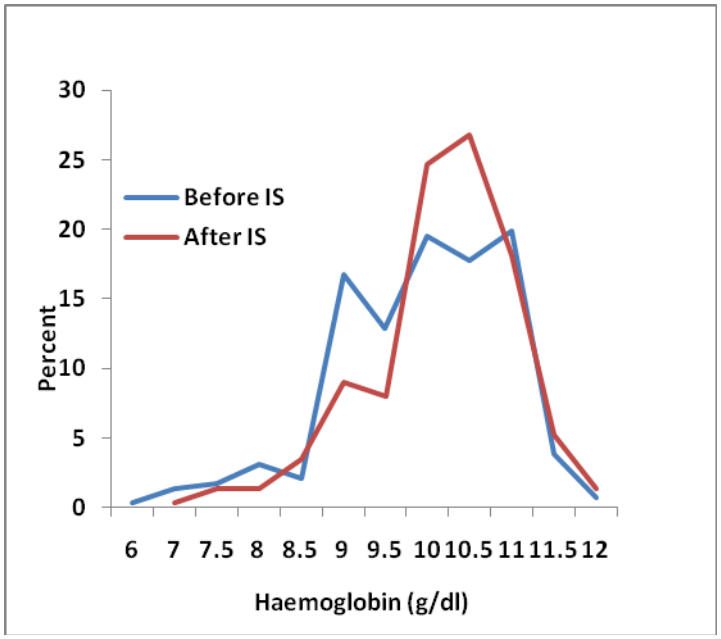
X axis: Clinical indicators catergories- Normal, pale very pale
Y axis: Haemoglobin g/dl

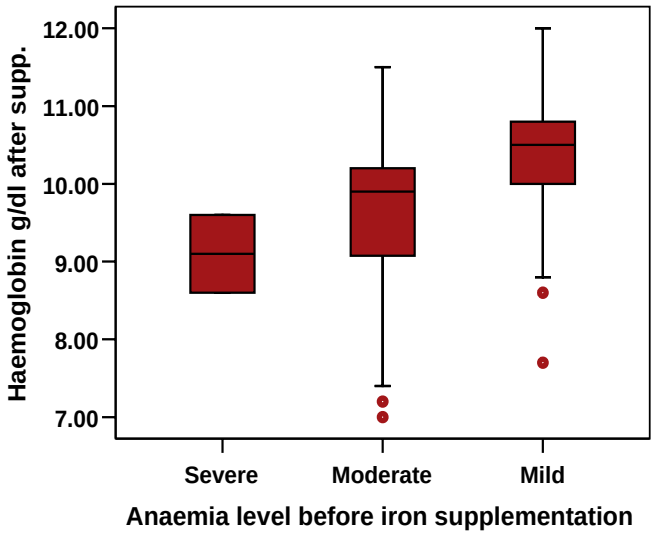
Figure 2: Box Plots of haemoglobin concentration (Median and Quartile Deviations) by pallor of clinical Indicators



A: Helliges Vs CYAN-MATH method
B: Clinical Indicators Vs CYAN-MATH method(Area under the cures are not significantly different among all of the clinic indicators)

Figure 3: ROC curves for the diagnosis of anaemia





(A: Frequency distribution in percent; B: Box plots of median and quartile deviations)

Figure 4: Haemoglobin concetration before and after iron supplementation