Diagnosing anaemia in pregnancy in rural clinics: assessing the potential of the Haemoglobin Colour Scale

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Anaemia in pregnancy is a common and severe problem in many developing countries. Because of lack of resources and staff motivation, screening for anaemia is often solely by clinical examination of the conjunctiva or is not carried out at all. A new colour scale for the estimation of haemoglobin concentration has been developed by WHO. The present study compares the results obtained using the new colour scale on 729 women visiting rural antenatal clinics in Malawi with those obtained by HemoCue haemoglobinometer and electronic Coulter Counter and with the assessment of anaemia by clinical examination of the conjunctiva. Sensitivity using the colour scale was consistently better than for conjunctival inspection alone and interobserver agreement and agreement with Coulter Counter measurements was good. The Haemoglobin Colour Scale is simple to use, well accepted, cheap and gives immediate results. It shows considerable potential for use in screening for anaemia in antenatal clinics in settings where resources are limited.

Voir page 20 le résumé en français. En la página 20 figura un resumen en español.

Introduction

In most developing countries anaemia in pregnancy makes an important contribution to maternal mortality and morbidity (1, 2). A haemoglobin concentration ([Hb]) of < 11.0 g/dl is commonly taken as indicative of anaemia in pregnancy (3).

Successful management of anaemia in pregnancy depends on accurate and acceptable methods of detecting anaemia, assessing its severity and monitoring response to treatment (4). In women with mild-to-moderate anaemia, timely treatment is likely to prevent the development of more severe anaemia and therefore reduce the need for blood transfusion with its associated risks. Prevention of severe anaemia also has more direct benefits for both mother and child.

In developed countries, where the prevalence of anaemia is below 20%, an accepted standard of practice is that all women have at least one measurement of [Hb] during the course of pregnancy. This is usually performed by electronic counter. In developing countries with reported prevalences of 40–70% these methods are often not available, even at the tertiary level. Screening for anaemia may not be carried out at all, or may be limited to inspection of the conjunctiva for the presence of pallor. There are no published reports of the accuracy of screening for anaemia using clinical inspection of conjunctiva

A new colour scale for assessing [Hb] has recently been developed by WHO (9). We have conducted a study to determine the value of this technique as a screening method for anaemia in rural antenatal clinics when used by local staff. The results are compared with values estimated for the same individuals by clinical examination of the conjunctiva and by measurements of [Hb] using a battery-operated HemoCue machine. As a standard for comparison, [Hb] measurements were obtained on venous blood samples using a Coulter Counter (Onyx, Coulter Counters, Johannesburg, South Africa).

Materials and methods

A total of 44 nurse—midwives from five different sites (three rural hospitals and two health centres) in southern Malawi each attended a one-day training session on the use of the HemoCue machine and the Haemoglobin Colour Scale. Training was given according to a standard format. As all staff were already familiar with conjunctival assessment no extra training was given in this method. All five sites were subsequently revisited three to four times each over a period of 3 months. At each antenatal clinic, three nurse—midwives independently assessed

alone in pregnant women in the rural antenatal clinic setting. Studies in children (\mathfrak{H}) and healthy ambulatory adults (\mathfrak{H}) have demonstrated poor accuracy. Even when used in combination with a conjunctival or anaemia recognition card, sensitivity remains low except when anaemia is severe (\mathcal{T} , \mathcal{H}). There is a need for a simple, cheap but accurate method for the estimation of haemoglobin concentration.

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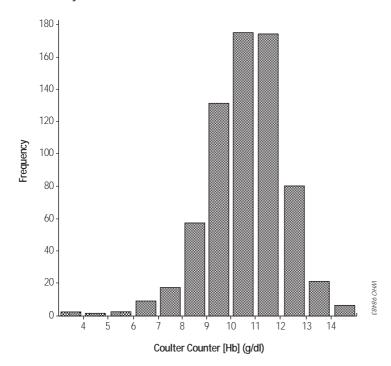
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whether the patient was anaemic, as follows: 1) inspection of the conjunctiva; 2) use of the colour scale; or 3) use of a battery-operated HemoCue machine. These tests were carried out in sequence from 1) to 3). The midwife carrying out each test had no knowledge of the results obtained using any of the other methods. When additional staff were available, more than one assessment using the colour scale was undertaken. All the women tested gave their free and informed consent. Results obtained by HemoCue were entered on the patients' antenatal records. The investigators had no previous knowledge of the antenatal client and no client had a prior [Hb] recorded on her antenatal card.

Inspection of conjunctiva. This was carried out by gently everting the lower eyelid and directly inspecting the ocular and palpebral conjunctiva. Results were graded as "pink", pale or very pale. Any assessment graded as pink indicated a perceived absence of anaemia, pale indicated the presence of anaemia, and very pale the presence of severe anaemia.

Colour scale. A finger-prick drop of blood was placed on a strip of absorbent paper. After disappearance of the sheen, the colour was compared with the set of six colour standards. The test strip was held behind the scale and the blood spot viewed through 8–9 mm apertures. Care was taken to hold the colour scale at an angle of about 45° in daylight with the light coming from behind the investigator. Investigators were instructed to compare from the bottom of the scale upwards. The [Hb] value recorded corresponded to the closest colour standard match. Colour standards on the scale correspond to haemoglobin values of 4, 6, 8, 10, 12 and 14 g/dl. This method has been well described by Stott & Lewis (9).

Fig. 1. Distribution of haemoglobin concentration measurements obtained by Coulter Counter.



HemoCue haemoglobinometer. The standard cuvette was filled with a drop of blood from the same finger-prick. After calibration of the machine, [Hb] values were read and recorded to one decimal point.

Coulter Counter: A venous sample was taken within minutes of the finger-prick test and transported in ethylenediaminetetraacetic acid (EDTA) tubes at 4 °C to a central laboratory at the Department of Obstetrics and Gynaecology, College of Medicine, Blantyre. Analysis by Coulter Counter was performed within 24 hours of sampling. [Hb] values obtained were recorded to 1 decimal point.

Statistical methods. All data were entered, verified, and analysed using SPSS and GENSTAT for Windows software. Sensitivity, specificity, accuracy, positive and negative predictive values and likelihood ratios were calculated for each of the methods and for the following [Hb] cut-off points: ≤ 11.0 g/dl, $\leq 10.0 \text{ g/dl}$, $\leq 8.0 \text{ g/dl}$ and $\leq 6.0 \text{ g/dl}$. The cut-off point of ≤ 11.0 g/dl was taken to reflect the WHO definition for anaemia in pregnancy. Other cut-off points were taken to comply with the intervals on the Haemoglobin Colour Scale and reflect different degrees of moderate anaemia ($\leq 10 \text{ g/dl}$, \leq 8 g/dl) and severe anaemia (\leq 6.0 g/dl). [Hb] as measured by Coulter Counter was taken as the "gold standard" against which other assessments were compared.

Sensitivity was calculated as true positives/(true positives + false negatives), specificity as true negatives/(true negatives + false positives), accuracy as (true positives + true negatives)/all tested, positive predictive value as true positives/(true positives + false positives), negative predictive value as true negatives/ (true negatives + false negatives) and likelihood ratio as sensitivity/(100 - specificity) (10-12).

The probability of diagnosing various degrees of anaemia as a function of haemoglobin concentration was modelled using logistic regression (13). Interobserver variability was assessed by weighted Kappa values (κ) with < 0.40 indicating poor to fair agreement, 0.40–0.60 moderate agreement, 0.60–0.80 substantial agreement and > 0.80 almost perfect agreement between assessors (van den Broek et al., unpublished results, 1998).

Results

A total of 729 women were recruited into the study. Only three declined to have a venous blood sample taken. A complete data set consisting of a conjunctiva examination, HemoCue and Coulter Counter measurement of [Hb] (g/dl) and at least one estimation using the Haemoglobin Colour Scale was available for 641 cases. In each clinic more than one investigator could use the colour scale and a total of 1066 observations are available for this method. In 10% of cases (107/1066) a value other than an even number (4, 6, 8, 10, 12 or 14) was reported, e.g. 5, 7, 11, 13. These values have not been modified in any way and are included in the analysis.

Conjunctiva assessments with matching Coulter Counter measurements were obtained in 644 cases and HemoCue and Coulter Counter measurements could be compared in 671 cases.

Fig. 1 shows the distribution of [Hb] measurements obtained by Coulter Counter in the population of 729 antenatal women examined. Percentage prevalences for categories of anaemia were 58.1% for [Hb] ≤ 11.0 g/dl, 32.0% for [Hb] ≤ 10.0 g/dl, and 4.0% for [Hb] ≤ 8.0 g/dl. Only three women had an [Hb] of < 6.0 g/dl and 49.5% of values obtained were in the range 10.0-11.9 g/dl.

Table 1 gives the calculated sensitivity, specificity, accuracy, positive and negative predictive values and likelihood ratios for the diagnosis of anaemia for four different cut-off points of [Hb] and for the three different methods tested in the field: HemoCue haemoglobinometer, conjunctival inspection and the colour scale. For the HemoCue, sensitivity was 80–96.6%, for conjunctival inspection 33.2–62.1% and for the Haemoglobin Colour Scale 50.0–81.6%. Positive predictive values were in the range 46.8-68.1% for the HemoCue, 1.2-75.0% for conjunctival examination and 11.0-66.2% for the colour scale. The highest sensitivity for the colour scale was obtained at a cut-off point of 10.0 g/ dl and the highest positive predictive value at 11.0 g/dl.

Logistic regression was used to model the influence of Coulter Counter values on the probability of the investigators' assessment of [Hb] being ≤ 8.0 and ≤ 10.0 g/dl. Regression lines are presented in Fig. 2. For values of [Hb] of ≤ 8.0 g/dl the regression line is defined by the expression ln(P/(1-P)) = 5.328 - 0.6133 CC (P = 0.5 when CC = 8.69 g/dl), and for values of [Hb] of ≤ 10.0 g/dl by ln(P/(1-P)) = 10.0

Table 2 shows the predicted proportions of subjects for various ranges of haemoglobin values for which the colour scale assessment is ≤ 8.0 g/dl and ≤ 10.0 g/dl using the logistic regression models fitted. Also shown are the proportions actually observed using the colour scale. As the [Hb] values increase, both the observed and predicted proportions generally decrease, as expected. For most [Hb] values there is fairly good agreement between these two sets of proportions; however, for high values, the observed proportions classified as having [Hb] ≤ 8.0 g/dl or ≤ 10.0 g/dl tend to be higher than predicted.

Agreement of the colour scale readings to within \pm 1 g/dl of the measurement obtained by Coulter Counter was obtained in 40% (429/1066) of cases and to within \pm 2 g/dl in 67% of cases (717/1066).

Interobserver variability for the colour scale was calculated using the results for the 334 subjects for whom two readings were obtained: 36% of readings were in exact agreement, (ordinary) = 0.177; agreement to within \pm 2 g/dl was obtained in 81%, (weighted) = 0.472 (n = 334).

Discussion

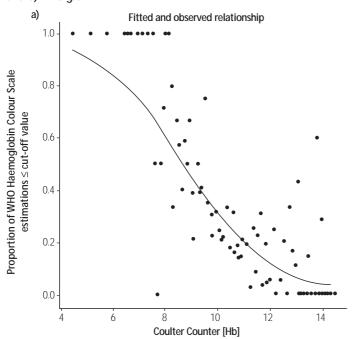
The diagnostic value of a test depends on its accuracy and its reliability. Accuracy can be determined

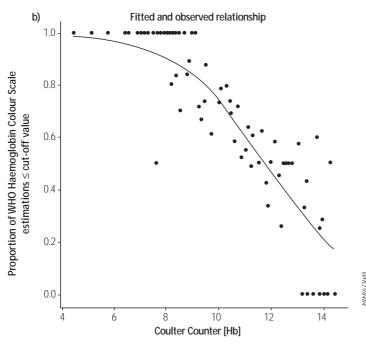
Table 1. Sensitivity, specificity, accuracy, positive and negative predictive values (PPV, NPV) and likelihood ratio (LR) for diagnosing anaemia at different cut-off points of haemoglobin concentration

Definition of anaemia (haemoglobin concentration in g/dl)	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)	LR
HemoCue						
≤11	85.4	80.1	81.4	56.7	94.7	4.3
≤10	94.0	79.1	83.9	68.1	96.5	4.5
≤8	96.6	94.6	94.9	46.8	99.8	17.8
≤6	80.0	99.5	99.4	57.1	99.8	160.0
Conjunctiva examination						
≤11	33.2	84.1	54.2	75.0	46.8	2.1
≤10	39.7	80.5	67.2	49.4	73.5	2.0
≤8	62.1	75.6	75.0	10.7	97.7	2.5
≤6	50.0	74.1	73.9	1.2	99.6	1.9
Colour scale						
≤11	75.4	47.2	63.5	66.2	58.2	1.4
≤10	81.6	45.3	56.8	40.8	84.2	1.5
≤8	81.1	76.4	76.5	11.0	99.1	3.4
≤6	50.0	98.5	98.2	15.8	99.7	33.3

by comparison with a suitable standard, in this case [Hb] measured by Coulter Counter. Reproducibility was assessed by measurement of interobserver variability. In addition, probability of diagnosing anaemia as a function of [Hb] was estimated using logistic regression analysis. For the purpose of screening an antenatal population for anaemia, high sensitivity is desirable since it is important that as many individuals as possible with anaemia have a positive test result (10). Subsequent management, e.g. prescription of iron tablets, is unlikely to be detrimental to those women who are overdiagnosed by the test used, i.e. the false positives.

Fig. 2. Logistic regression lines modelling the influence of Coulter Counter haemoglobin concentration on the probability of assessment as anaemic using the Haemoglobin Colour Scale cut-off values of a) \leq 8 g/dl; and b) \leq 10 g/dl.





Accuracy and consistency of the Haemoglobin Colour Scale

Previous studies evaluating the diagnostic accuracy of the Tallqvist colour scale have reported it to be insufficiently reliable when used in the field. At a cut-off point of 10 g/dl the Tallqvist scale had a sensitivity of 60.5% and a positive predictive value of 46.0% when used in an urban clinic by trained health aides (13). The new Haemoglobin Colour Scale was designed to overcome some of the causes of inaccuracy in older colour scales (9). New colour standards were developed and a new booklet prepared with sufficiently large apertures on the test card. Preliminary evaluation under laboratory conditions showed a good correlation with Coulter Counter measurements. Values obtained for sensitivity were in the range 80.0–95.2% and the positive predictive value was 63.0–98.5% (14).

Even for less subjective methods, accuracy decreases considerably in actual usage as compared to laboratory evaluation and it is important to evaluate equipment intended for primary health within the local setting (15). Values obtained in this field study are not as high as for laboratory evaluation but sensitivity is consistently better than for conjunctival inspection alone and, except for a cut-off point of ≤ 6.0 g/dl, is also better than previously reported for a filter-paper method. The specificity is no doubt influenced by the distribution of [Hb] values obtained; 50% of women had values in the range 10.0-11.9 g/dl. Similarly, positive predictive values would have been higher if the prevalence of severe anaemia had been higher than observed in the study population. Nevertheless this study provides a more accurate reflection of performance of the test under field conditions than evaluation under laboratory conditions. Secondly, the [Hb] distribution in this antenatal study population is similar to that reported from other developing countries and the performance of the methods we examined can be expected to be similar. As the severity of anaemia increases, diagnostic accuracy improves. None of our investigators had any previous experience in measuring [Hb] other than by assessing conjunctival pallor. Accuracy may presumably be improved further with experience in use and repeated training where the investigator is more aware of misclassification errors and differences in colour perception. Further studies are necessary to determine this.

Given that the colour standards on the Haemoglobin Colour Scale are in 2-g/dl gradations, 81% agreement between assessors to within \pm 2 g/dl and 67% agreement to within \pm 2g/dl of the Coulter Counter measurement is good. Disagreement with the Coulter Counter value of more than \pm 2g/dl was noted in 15.5% (165/1066) of samples. Of these, 44% (73/165) were from the most distant study site and, although Coulter Counter readings were obtained within 24 hours, the interval between sampling and measurement was clearly longer than for samples obtained closer to the laboratory. Storage and transport of samples may have affected the read-

ings. To reflect field conditions, we have not excluded these values from our analysis, but had we done so the accuracy of the scale could be expected to be better than indicated here.

Other simple screening methods

As mentioned earlier, accuracy is low when anaemia is diagnosed by conjunctival inspection alone. For under-5-year-olds, a definite diagnosis of anaemia was correctly made in 5-42% of cases and a diagnosis of probable anaemia was correctly made in 24-64% of cases by conjunctival inspection (5). When palm, tongue and nailbed were examined in addition, accuracy improved. However, in this paediatric study a low [Hb] cut-off point of 8 g/dl was used to define anaemia. Similarly, Gjorup et al. obtained a sensitivity of 27-44% at cut-off points of 10.0 g/ dl and 11.0 g/dl, and the expected probability of detecting anaemia did not exceed 0.75 even when inspection was carried out by trained physicians (12). Substantial interobserver variability has also been reported (6, 12, 16, 17). In the current study, sensitivity was 33.2% and 39.7% for [Hb] values of \leq 11.0 and \leq 10.0 g/dl, respectively. Values obtained were better for the lower range of [Hb] values but did not exceed 62.1%. Conjunctival inspection in pregnant women may be particularly inaccurate as a result of increased peripheral vasodilatation. It may be possible to improve the accuracy of screening for anaemia using conjunctival inspection in pregnant women by improved training and assessment of more than one physical sign of pallor. However, in our study no extra training in this method was given to the investigators.

The HemoCue method had excellent sensitivity and specificity at all cut-off levels of [Hb] tested. Values obtained were comparable with those previously reported for field studies (88.5% sensitivity and 77.6% specificity) (15). It must be noted that a small bias is possible when the results of finger-prick samples (HemoCue) are compared with those of a venous blood sample (Coulter Counter). The HemoCue method was well liked by health care workers but in practice it is still too expensive for use in the primary health care setting in most developing countries.

Recently the use of the copper sulfate (18, 19) and undiluted Lovibond (20, 21) methods has been re-evaluated and recommended for screening purposes. The values obtained for sensitivity with the Haemoglobin Colour Scale (50–81.6%) are comparable to those reported for these methods (75.8–87.5% and 87.4%, respectively), both of which require standard solutions and/or standardized equipment.

Operational and logistic appeal

The Haemoglobin Colour Scale is simple to use, cheap, and gives an immediate result. Health care workers appreciated having a method which gave an actual measurement of [Hb] as opposed to in-

spection of conjunctiva with categories of pink, pale or very pale only (van den Broek et al., unpublished results, 1998). Patients were curious to see their "colour of blood" a phrase which is commonly used to explain anaemia in developing countries. Hence compliance with the test was excellent. A better understanding of the concept of anaemia by pregnant women is likely to lead to improved compliance with prophylactic or therapeutic measures, such as iron tablets.

Potential clinical usefulness

Screening for anaemia in pregnancy is useful for a variety of reasons. It may be helpful to collect baseline data on prevalence and severity in a given population, and to assess the effects of supplementation with iron tablets, antimalarial prophylactics or antihelminthic treatment. At primary care level, estimation of [Hb] can help decide whether referral is necessary for more detailed investigation and treatment.

The value of each screening test within a specific setting depends on the necessity of performing an assessment in the absence of a more accurate method and on cost-benefit considerations. Any method of screening for anaemia at primary health care level in a developing country should be acceptable to both patients and staff, simple to operate, require a minimum of materials, be cheap and give immediate accurate results. In situations with limited resources and poor technical support, a simple screening tool is likely to perform better than sophisticated methods that depend on correct dilution and preparation of standards or on power supply. From this perspective, the Haemoglobin Colour Scale has considerable potential as an exciting new tool for use in antenatal clinics. Further field testing is necessary to evaluate whether use of the colour scale can permit health staff to detect the effect of therapy, be similarly successful in recognition and management of anaemia in other patient groups, and allow the identification of potential blood donors.

Table 2. Observed and predicted proportions of Coulter Counter readings (n = 1066) correctly classified as haemoglobin concentrations [Hb] of ≤ 8.0 g/dl and ≤ 10.0 g/dl by the Haemoglobin Colour Scale (logistic regression analysis)

Coulter Counter		Colour sca	le ≤8.0 g/dI	Colour scale ≤10.0 g/dl		
[Hb] (g/dl)	n	Predicted	Observed	Predicted	Observed	
3.5-4.4	2	0.947	1.000	0.990	1.000	
4.5 - 5.4	2	0.906	1.000	0.982	1.000	
5.5-6.4	4	0.839	1.000	0.968	1.000	
6.5 - 7.4	11	0.738	0.909	0.944	1.000	
7.5-8.4	50	0.604	0.700	0.903	0.900	
8.5-9.4	130	0.452	0.438	0.837	0.831	
9.5-10.4	256	0.309	0.273	0.739	0.750	
10.5-11.4	291	0.195	0.165	0.609	0.588	
11.5-12.4	234	0.116	0.128	0.462	0.462	
12.5-13.4	55	0.066	0.182	0.322	0.436	
13.5–14.4	31	0.037	0.161	0.207	0.226	

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Résumé

Diagnostic de l'anémie chez la femme enceinte dans les dispensaires ruraux : évaluation du potentiel de la nouvelle échelle colorée

L'anémie chez la femme enceinte est courante dans de nombreux pays en développement. Au niveau des soins de santé primaires, le dépistage de l'anémie, quand il est pratiqué, se limite souvent à l'examen clinique de la conjonctive. L'OMS a mis au point une nouvelle échelle colorée pour déterminer l'hémoglobinémie (échelle de coloration pour le dosage de l'hémoglobine). Nous avons formé à son emploi les agents de santé des dispensaires ruraux de soins prénatals dans le sud du Malawi et effectué une étude de terrain auprès de 729 femmes venues dans ces dispensaires pour évaluer sa précision diagnostique, sa fiabilité et son acceptabilité en tant que méthode de dépistage de l'anémie dans cette population. Les dosages de l'hémoglobine par l'échelle de coloration ont été comparés aux valeurs obtenues au moyen d'un hémoglobinomètre à piles HemoCue et à l'évaluation de l'anémie par l'examen clinique de la conjonctive. Pour chaque sujet, une mesure au Coulter Counter a été effectuée sur un prélèvement de sang veineux et utilisée comme étalon aux fins de comparaison. Les résultats sont présentés pour les seuils [Hb] $\leq 11,0$ g/dl, $\leq 10,0$ g/dl, $\leq 8,0$ g/dl et $\leq 6,0$ g/dl. Pour chaque seuil, la sensibilité était systématiquement plus élevée pour l'échelle de coloration (50,0%–81,6%) que pour l'examen clinique de la conjonctive (33,2%–62,1%). L'influence de la distribution des taux de [Hb] dans la population étudiée sur les valeurs diagnostiques est examinée. On a calculé par régression logistique les proportions observées et prévues des mesures obtenues par le Coulter Counter, classées en fonction des seuils [Hb] < 8,0 g/dl et < 10,0 g/dl, ainsi que l'influence de [Hb] sur la probabilité d'un diagnostic d'anémie. Dans 67% des cas, les mesures obtenues au moyen de l'échelle colorée de l'OMS correspondaient à ± 2 g/dl près à la mesure enregistrée par le Coulter Counter. L'intérêt clinique et logistique de la nouvelle échelle de coloration est examiné. L'échelle de coloration est simple à utiliser, bien acceptée, bon marché et elle donne des résultats immédiats. Elle paraît très prometteuse pour le dépistage de l'anémie dans les dispensaires prénatals là où les ressources sont limitées.

Resumen

Diagnóstico de la anemia gestacional en los dispensarios rurales: evaluación del potencial de la nueva escala cromática para la estimación de la hemoglobina

La anemia durante el embarazo es un problema común en muchos países en desarrollo. A nivel de la atención primaria, la detección de la anemia, cuando se hace, suele consistir en la inspección clínica de la conjuntiva solamente. La OMS ha ideado una nueva escala cromática para estimar la concentración de hemoglobina en la sangre ([Hb]), cuyo uso se ha enseñado a los agentes de salud de los dispensarios rurales de atención prenatal del sur de Malawi. Para determinar su grado de exactitud de diagnóstico, fiabilidad y aceptabilidad como método de detección de la anemia se hizo un estudio sobre el terreno con 729 mujeres de esos dispensarios. Las estimaciones de [Hb] con la escala cromática se compararon con las mediciones obtenidas con el hemoglobinómetro HemoCue y con los resultados del examen clínico de la conjuntiva. A partir de una muestra de sangre venosa de las pacientes se obtuvieron valores de referencia con el contador de Coulter para fines de comparación. Los resultados se presentan para valores límite de [Hb] de $\leq 11,0, \leq 10,0,$ \leq 8,0 y \leq 6,0 g/dl. Respecto de cada valor límite, la

sensibilidad fue sistemáticamente mayor en el caso de la escala cromática (50,0%-81,6%) que en el del examen clínico de la conjuntiva (33,2%–62,1%). El efecto que la distribución de los valores de [Hb] en la población estudiada tiene sobre los valores de diagnóstico obtenidos es objeto de discusión. Se utilizó la regresión logística para calcular las proporciones observadas y predichas de los datos obtenidos con el contador de Coulter clasificados correctamente como [Hb] \leq 8,0 y ≤ 10,0 g/dl, así como la influencia de la [Hb] sobre la probabilidad de que se diagnostique anemia. En el 67% de los casos hubo correspondencia, dentro de un margen de \pm 2 g/dl, entre las lecturas de la escala cromática y la medición registrada por el contador de Coulter. Se examinan la posible utilidad clínica y el interés logístico de la nueva escala cromática, que es fácil de utilizar, tiene buena aceptación, es barata y da resultados inmediatos. Sus posibilidades de uso para detectar la anemia en los dispensarios prenatales con recursos limitados son considerables.

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