Estimating the prevalence of anaemia: a comparison of three methods

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Objective To determine the most effective method for analysing haemoglobin concentrations in large surveys in remote areas, and to compare two methods (indirect cyanmethaemoglobin and HemoCue) with the conventional method (direct cyanmethaemoglobin).
Methods Samples of venous and capillary blood from 121 mothers in Indonesia were compared using all three methods.
Findings When the indirect cyanmethaemoglobin method was used the prevalence of anaemia was 31–38%. When the direct cyanmethaemoglobin or HemoCue method was used the prevalence was 14–18%. Indirect measurement of cyanmethaemoglobin had the highest coefficient of variation and the largest standard deviation of the difference between the first and second assessment of the same blood sample (10–12 g/l indirect measurement vs 4 g/l direct measurement). In comparison with direct cyanmethaemoglobin measurement of venous blood, HemoCue had the highest sensitivity (82.4%) and specificity (94.2%) when used for venous blood.
Conclusions Where field conditions and local resources allow it, haemoglobin concentration should be assessed with the direct cyanmethaemoglobin method, the gold standard. However, the HemoCue method can be used for surveys involving different laboratories or which are conducted in relatively remote areas. In very hot and humid climates, HemoCue microcuvettes should be discarded if not used within a few days of opening the container containing the cuvettes.

Keywords Hemoglobinometry/methods; Anemia/epidemiology; Prevalence; Comparative study; Indonesia (*source: MeSH*).

Mots clés Hémoglobinométrie/méthodes; Anémie/épidémiologie; Prévalence; Etude comparative; Indonésie (*source: INSERM*).

Palabras clave Hemoglobinometría/métodos; Anemia/epidemiología; Prevalencia; Estudio comparativo; Indonesia (*fuente: BIREME*)

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Introduction

Anaemia, defined as a reduced haemoglobin concentration, is associated with increased perinatal mortality, increased child morbidity and mortality, impaired mental development, impaired immune competence, increased susceptibility to lead poisoning, and decreased performance at work (*1–4*). To identify the most likely cause of anaemia, such as low iron intake or infection, distribution curves of the haemoglobin concentration of different groups within a population can be compared (*5*).

In the developing countries of south-east Asia the prevalence of anaemia among pregnant women is as high as 60–70% (*5*). In Indonesia, a national survey in 1992 found that 64% of pregnant women and 56% of children under 5 years old were anaemic (*6*); a household survey in 1995 found that 51% of pregnant women and 41% of children under 5 years had anaemia (*6*). About 30% of female workers and 24–35% of schoolchildren also were anaemic (*7*).

Estimates of the prevalence of anaemia depend on the methods used for assessing haemoglobin concentration and on the cut-off point applied: the cut-off point is different for different groups in a population (*8, 9*). For a prevalence survey, the choice of method for measuring haemoglobin concentration

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and the decision whether to test venous or capillary blood depend not only on the performance of the method but also on the conditions under which the blood will be collected, such as the remoteness of the location, whether laboratory support is available, whether staff are experienced, and whether the study population is cooperative.

Direct cyanmethaemoglobin measurement: the gold standard

The gold standard for assessing haemoglobin concentration is the direct cyanmethaemoglobin method. However, this method requires that a laboratory with a spectrophotometer is available within a few hours' travelling time from where the blood is collected and, to avoid inter-laboratory variability, it is necessary that all measurements are conducted by the same laboratory. Thus, a different method is usually required for surveys that are conducted over a comparatively large area and/or in remote conditions. One possible method is the indirect measurement of cyanmethaemoglobin, for which blood is dried on filter-paper for transport and then redissolved at the laboratory for measurement. Another method uses a new generation of haemoglobin photometer, the HemoCue (HemoCue, Angelhom, Sweden). The HemoCue method can be used in the field to analyse blood collected in a microcuvette. The photometer is easy to transport because it is small and light; it is battery operated and gives consistent results (*10*).

The aim of this study was to determine which of these two alternative methods for determining haemoglobin concentration (indirect measurement of cyanmethaemoglobin or HemoCue) is more useful in large surveys in remote areas and which method performs better when compared with the direct measurement of cyanmethaemoglobin. Each method was used for both venous and capillary blood collected from the same participants.

Methods

Participants

This study was part of an end-line survey for monitoring the impact of a social marketing project in the area of the city of Banjarmasin that is under the governance of South Kalimantan. The study was carried out by Helen Keller International Indonesia in collaboration with the provincial health office in South Kalimantan in March and April 1998. The participants were 121 mothers who had children aged under 5 years of age and who had participated in the end-line survey. Of these 121 mothers, 62 were in Banjarmasin and 59 were in Martapura (another city in South Kalimantan), the control area for the assessment of the social marketing campaign. Written informed consent was obtained from the participants after explaining the aim of the blood collection. The procedure for blood collection was approved by the Medical Ethical Committee of the Indonesian Ministry of Health.

Study design

Haemoglobin concentration was determined in six different ways for each participant. Both venous and capillary blood samples were collected, and the haemoglobin concentration of each sample was determined using direct measurement of cyanmethaemoglobin (the conventional spectrophotometry method), the indirect method (blood was dried on a paper-filter to preserve it for analysis in the laboratory 2 weeks after collection), and the HemoCue method.

Blood collection

Dry syringes were used to collect 3 ml of venous blood from the forearm. The blood was transferred to a glass tube and divided for assessment by the different methods. For capillary blood, the participant's fingertip was warmed, cleaned with alcohol, and punctured with a needle using an Autoclix apparatus. The first drop of blood was discarded; blood was then collected for the three methods using a Sahli pipette twice and a HemoCue microcuvette.

For both the direct and indirect cyanmethaemoglobin methods, duplicate measurements were made from the same blood sample. For the HemoCue method, only one measurement was carried out for capillary blood; for venous blood, two assessments were made, using an old and new microcuvette.

Analysis of blood samples

Anaemia was defined as haemoglobin concentration <120 g/l, as assessed by the direct method on venous blood. Direct cyanmethaemoglobin measurement of venous blood was used as the gold standard for determining the sensitivity and specificity of the other methods. Variability within samples was calculated from duplicate measurements (direct and indirect cyanmethaemoglobin methods only). Systematic differences between the methods were assessed using samples from the same participant. For each participant, the difference between two methods was calculated and those differences were averaged at group level to assess whether they were different from zero.

Direct and indirect cyanmethaemoglobin methods. For the direct method, 20 µl blood were mixed with 5 ml Drabkin's solution in the field. After 2–4 hours the blood was analysed at the local laboratory by experienced technicians using a spectrophotometer. For the indirect cyanmethaemoglobin method, 20 µl blood was transferred onto Whatman filter-paper and dried at room temperature. After the blood had dried, the filter-paper was put into a small plastic bag (one sample per bag), which was placed in an envelope and sealed. The filter-papers were taken to the laboratory at the Nutrition Research and Development Center in Bogor, where they were analysed 2 weeks after being collected. The dried blood was then diluted in 5 ml Drabkin's solution, and the

Table 1. **Comparison of different methods for assessing haemoglobin (Hb) concentration in blood samples (n = 121)**

Parameter	Direct cyanmethaemoglobin		Indirect cyanmethaemoglobin		HemoCue	
	Venous	Capillary	Venous	Capillary	Venous	Capillary
Mean[a] Hb concentration g/l	130.1 (†) (‡)	128.6 (†)	127.4 (†)	123.4 (§)	131.9 (‡)	131.9 (‡)
SD[b] of the mean g/l	11.4	12.0	14.5	12.7	13.7	12.0
% of participants with Hb concentration: <110 g/l	4.9	6.6	9.9	12.4	6.6	5.8
110–119 g/l	9.1	11.6	21.5	25.6	9.9	8.2
⩾120 g/l	86.0	81.8	68.6	62.0	83.5	86.0
Coefficient of variation (%)	8.76	9.33	11.38	10.29	10.39	9.10
Sensitivity[c] (%)	NA[e]	94.1	76.5	76.5	82.4	70.6
Specificity[d] (%)	NA[e]	94.2	76.0	68.3	94.2	95.2

[a] Mean values with a different symbol in parentheses (†, ‡, §) were significantly different from each other, $P < 0.05$ (ANOVA with post-hoc test for least significant differences).

[b] SD = standard deviation.

[c] Proportion of anaemic participants detected by a particular method.

[d] Proportion of non-anaemic participants detected by a particular method.

[e] NA = not applicable. Direct cyanmethaemoglobin measurement of venous blood was the standard against which other methods were judged.

haemoglobin concentration was determined using a spectrophotometer. Both direct and indirect determinations used the Merck-test 3317 (Merck, Darmstadt, Germany) for determining haemoglobin concentration.

HemoCue method. For the HemoCue method, one drop of blood was collected in a HemoCue microcuvette and the haemoglobin concentration was read directly in the field. As the HemoCue cuvettes are very hygroscopic, we compared the results when using cuvettes from a container that had been opened on the day the sample was taken with those obtained using cuvettes from a container that had been opened 2–25 days before use.

Statistical analysis

Data were entered using FoxPro 2.6 for Windows and were converted to SPSS 7.5 for Windows for analyses. Results from duplicate measurements for the direct and indirect method were averaged and the haemoglobin concentration was reported as the mean along with the standard deviation.

Results

The average age of the 121 participants was 29.2 years. Almost 40% of the mothers had attended secondary school or had had higher education. The mean body mass index was 21.9 kg/m^2; however, in 15.7% of participants the body mass index was <18.5 kg/m^2. The prevalence of anaemia using direct assessment of venous blood was 14%.

Intra-sample variability

The standard deviation (SD) for the difference between the first and the second measurement of the same sample using the direct cyanmethaemoglobin method was 4.1 g/l for venous and 3.9 g/l for capillary blood, while for the indirect measurement the SD was 11.6 g/l for venous and 10.3 g/l for capillary blood (data not shown).

Comparison of haemoglobin concentration using the three methods

A summary of the results is shown in Table 1. The highest mean haemoglobin concentration was found using the HemoCue. The coefficient of variation was lowest for the direct method using venous blood and highest for the indirect assessment of venous blood.

The prevalence of anaemia ranged from 14.0% (when the direct method was used to assess venous blood) to 38.0% (indirect assessment of capillary blood); the prevalence of anaemia was significantly higher when using the indirect method for both type of blood samples. The prevalence of anaemia was comparable when assessed using the HemoCue and the direct method for both types of blood samples.

Sensitivity and specificity. Table 1 shows the sensitivity and specificity of the different methods in detecting anaemic participants. The sensitivity of direct assessment of capillary blood (94.1%) and the HemoCue assessment of venous blood (82.4%) was higher than that the other methods (70.6% for HemoCue for capillary blood and 76.5% for indirect assessment of either venous or capillary blood). Specificity was highest for the HemoCue assessment

of capillary (95.2%) or venous (94.2%) blood, and for direct assessment of capillary blood (94.2%). The specificity of the indirect method was relatively low (68.3% for capillary and 76% for venous blood).

Table 2 shows the correlation coefficients of the results of comparisons between direct assessment of venous blood and other methods. The correlation between the direct assessment of venous blood and the indirect assessment of venous or capillary blood was lower than that between direct assessment of venous blood and the HemoCue method for both types of blood samples. Table 2 also shows the percentage of participants for whom the difference between two methods (the direct cyanmethaemoglobin assessment of venous blood and another specified method) divided by the mean of two methods was less than 5%. The proportion with less than 5% difference was higher for comparisons with direct cyanmethaemoglobin assessment of capillary blood and the HemoCue assessment of venous and capillary blood.

HemoCue: comparison of new and old microcuvettes

The HemoCue assessment of venous blood was done twice: once using a microcuvette from a container whose seal had been broken on the day of the measurement and once with a microcuvette from a container whose seal had been broken 2–25 days before the measurement. Table 3 shows the results of this comparison. The mean haemoglobin concentration as assessed with old cuvettes was 2.7 g/l (2.3%) higher than that assessed with new cuvettes. A closer analysis of the data showed that there was no difference between the old and new cuvettes when the old cuvettes came from a container whose seal had been broken no more than 12 days before the measurement. Cuvettes opened more than 12 days before the measurement detected significantly higher haemoglobin concentrations than new cuvettes (data not shown). The prevalence of anaemia as assessed with new and old (2–25 days old) cuvettes was significantly different ($P < 0.05$). The sensitivity of the old cuvettes (2–25 days old) for detecting anaemia in participants was 60.0% and the specificity was 96.0%.

Table 2. **Comparison of haemoglobin concentration in samples from the same participant ($n = 121$) as assessed by direct cyanmethaemoglobin measurement of venous blood and other methods**

Method	Correlation coefficient[a]	Mean paired difference[b] g/l	% of participants for whom the difference between 2 methods[c] divided by mean of 2 methods < 5%
Direct cyanmethaemoglobin measurement of capillary blood	0.946	–1.5 (–2.2 to –0.8)	65.3
Indirect cyanmethaemoglobin measurement of venous blood	0.625	–2.7 (–4.8 to –0.6)	36.4
Indirect cyanmethaemoglobin measurement of capillary blood	0.635	–6.8 (–8.6 to –4.9)	29.8
HemoCue measurement of venous blood	0.917	1.8 (0.8 to 2.8)	60.3
HemoCue measurement of capillary blood	0.848	1.8 (0.6 to 3.0)	52.1

[a] Pearson's correlation coefficient; $P < 0.05$ for all comparisons.

[b] Calculated as [haemoglobin concentration of sample calculated by method specified] minus [haemoglobin concentration of venous blood calculated by direct cyanmethaemoglobin method]. The figures in parentheses are 95% confidence intervals.

[c] For each participant, the results from each method were compared to those obtained by direct cyanmethemoglobin measurement of venous blood.

Table 3. **Results using HemoCue[a] with old and new microcuvettes ($n = 121$)**

Parameter	Old[b] cuvette	New[c] cuvette
Mean haemoglobin concentration[d] g/l (SD[e])	134.6 131.9	(13.7) (13.7)
Anaemia prevalence (%)	13.2	16.5
Sensitivity (%)	60.0	82.4
Specificity (%)	96.0	94.2

[a] HemoCue assessment of venous blood.

[b] Cuvette from a container opened 2–25 days before use.

[c] Cuvette from a container opened on the day of use.

[d] Paired difference = 2.7 $\pm$ 8.6 g/l ($P < 0.05$).

[e] SD = standard deviation.

Discussion

We found that the prevalence of anaemia was twice as high when the indirect method was used (31–38%) and compared with the results of the direct method or the HemoCue (14–18%). This may have been caused by the blood on the filter-paper being incompletely dissolved, although the technicians soaked the paper in Drabkin's solution until no stains were visible on it. The large difference in the estimated prevalence of anaemia determined using two different methods means that methodological differences should be examined critically before the results of different surveys are compared.

The haemoglobin concentration assessed in capillary blood was slightly lower than that assessed in venous blood, except when the HemoCue was used: when using HemoCue the results were the same. Because the capillary blood vessel is small, the red-cell volume of capillary blood is 1–3% lower than that of venous blood, and therefore its haemoglobin concentration is lower (*10*). The HemoCue, however, cannot detect such a small difference.

The method of choice for evaluating anaemia in remote areas would be the HemoCue method to assess venous blood; our second choice would be the HemoCue assessment of capillary blood; our third choice would be to the direct cyanmethaemoglobin method of assessing capillary blood. These choices were made using the comparisons of the sensitivity

and specificity of the different methods (Table 1). Given our experience of using these methods in the field, we would not recommend the use of the indirect cyanmethaemoglobin method because it identified a much higher prevalence of anaemia.

A comparison of the results obtained with the HemoCue using microcuvettes taken from a container opened on the day of the test and using those taken from a container that had been opened 2–25 days earlier showed that 1–2 weeks after breaking the seal the haemoglobin concentration was assessed as too high. Two studies from Indonesia have reported that unreliable data were obtained when cuvettes were used from a container on which the seal had been broken more than a couple of days before the measurement (M. Gliwitzki, personal communication, 1996) (*11*). In both of those studies, as well as in the study presented here, the containers were closed immediately after taking out a few cuvettes because of their hygroscopic nature. Thus, the manufacturer's statement that cuvettes should be used within 2 months of breaking the seal does not seem to hold for humid climates. In areas where there is high humidity, the 50 cuvettes contained in one container should be used within a few days of breaking the seal.

This study found that the prevalence of anaemia was overestimated by the indirect assessment method. Thus, where field conditions and local resources allow it, haemoglobin concentration should be assessed using the gold standard, the direct cyanmethaemoglobin method. However, the HemoCue is appropriate for surveys that are conducted over comparatively large areas, for those that require the use of different laboratories, or for those that take place in remote areas where adequate laboratory facilities are not available. The HemoCue is easy to use in the field: it is battery operated and there is little inter-observer error. However, the HemoCue costs a considerable amount of money, and this should be considered when planning and budgeting for data collection. ■

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Conflicts of interest: none declared.

Résumé

Estimation de la prévalence de l'anémie : comparaison entre trois méthodes

Objectif Déterminer quelle est la méthode la plus efficace pour mesurer la concentration en hémoglobine lors d'enquêtes à grande échelle réalisées dans des régions reculées, et comparer deux méthodes (mesure indirecte de la cyanméthémoglobine et HemoCue) avec la méthode classique (mesure directe de la cyanméthémoglobine).

Méthodes Des prélèvements de sang veineux et capillaire ont été réalisés en Indonésie chez 121 mères et les résultats obtenus par les trois méthodes ont été comparés.

Résultats Lorsqu'elle était déterminée par mesure indirecte de la cyanméthémoglobine, la prévalence de l'anémie était de 31-38 %. Par mesure directe de la cyanméthémoglobine ou par la méthode HemoCue, elle était de 14-18 %. La mesure indirecte de la cyanméthémoglobine donnait le plus fort coefficient de variation et le plus grand écart type de la différence entre la première et la deuxième mesure du même échantillon de sang (10-12 g/l avec la mesure indirecte contre 4 g/l avec la mesure directe). Par comparaison avec la mesure directe de la cyanméthémoglobine sur le sang veineux, la meilleure sensibilité (82,4 %) et la meilleure spécificité (94,2 %) revenaient à la méthode HemoCue appliquée au sang veineux.

Conclusion Lorsque les conditions de travail sur le terrain et les ressources locales le permettent, la concentration en hémoglobine doit être estimée par la méthode de mesure directe de la cyanméthémoglobine, qui est la méthode de référence. La méthode HemoCue peut toutefois être utilisée dans les enquêtes faisant appel à différents laboratoires ou réalisées dans des régions relativement reculées. En climat particulièrement chaud et humide, les microcuves HemoCue doivent être jetées si elles ne sont pas utilisées dans les quelques jours qui suivent l'ouverture du récipient.

Resumen

Estimación de la prevalencia de anemia: comparación de tres métodos

Objetivo Determinar el método más eficaz para analizar las concentraciones de hemoglobina en grandes encuestas llevadas a cabo en zonas remotas, y comparar dos métodos (cianometahemoglobina indirecta y HemoCue) con el método convencional (cianometahemoglobina directa).

Métodos Se compararon los resultados obtenidos con los tres métodos en muestras de sangre venosa y capilar de 121 madres de Indonesia.

Resultados El método de la cianometahemoglobina indirecta arrojó una prevalencia de anemia del 31%-38%. Con el método de la cianometahemoglobina

directa y con HemoCue, la prevalencia fue del 14%-18%. La medición indirecta de la cianometahemoglobina fue el método que presentó el coeficiente de variación más alto y la mayor desviación estándar de la diferencia entre la primera y la segunda evaluación de la misma muestra de sangre (10-12 g/l con el método indirecto, frente a 4 g/l con el método directo). Comparado con la determinación directa de la cianometahemoglobina en sangre venosa, HemoCue presentó la mayor sensibilidad (82,4%) y especificidad (94,2%) cuando se aplicó a la sangre venosa.

Conclusión Si las condiciones sobre el terreno y los recursos locales lo permiten, la concentración de hemoglobina debería determinarse con el método de la cianometahemoglobina directa, que sería la prueba de referencia. Sin embargo, el método HemoCue puede emplearse en las encuestas realizadas con la participación de diferentes laboratorios o en zonas relativamente remotas. En los climas muy cálidos y húmedos, una vez abierto el recipiente en que se suministran, las microcubetas de HemoCue no utilizadas al cabo de unos pocos días deben ser desechadas.

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