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Evaluation of two methods to measure hemoglobin concentration among women with genetic hemoglobin disorders in Cambodia: A method-comparison study

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ABSTRACT

Background: Genetic hemoglobin (Hb) E variants are common in Cambodia and result in an altered and unstable Hb molecule. We evaluated two methods to measure Hb concentration among individuals with and without Hb variants using a hemoglobinometer (HemoCue®) and a hematology analyzer (Sysmex XT-1800i).
Methods: We determined the bias and concordance between the methods among 420 Cambodian women (18–45 y).
Results: Bias and concordance appeared similar between methods among women with no Hb disorders ($n = 195$, bias $= 2.5$, $\rho_c = 0.68$), women with Hb E variants ($n = 133$, bias $= 2.5$, $\rho_c = 0.78$), and women with other Hb variants ($n = 92$, bias $= 2.7$, $\rho_c = 0.73$). The overall bias was 2.6 g/l, resulting in a difference in anemia prevalence of 11.5% (41% using HemoCue® and 29.5% using Sysmex, $p < 0.001$). Based on visual interpretation of the concordance plots, the HemoCue® device appears to underestimate Hb concentrations at lower Hb concentrations and to overestimate Hb concentrations at higher Hb concentrations (in comparison to the Sysmex analyzer).
Conclusions: Bias and concordance were similar across groups, suggesting the two methods of Hb measurement were comparable. We caution field staff, researchers and policy makers in the interpretation of data and the impact that bias between methods can have on anemia prevalence rates.

1. Introduction

Anemia is a serious public health problem affecting over 1.6 billion people worldwide, which is almost one quarter of the world's population [1]. Anemia is defined as a low hemoglobin (Hb) concentration and Hb cut off levels vary among populations based on multiple factors such as age, sex and altitude level [2]. Hb concentration is one of the most commonly measured indicators of health and nutrition. Anemia prevalence rates among populations can have strong policy and programming implications for the treatment, prevention and management of anemia, as the condition has serious health consequences for women [3–5] and children [6,7]. In laboratory settings, Hb can be measured in a sample of blood using an automated hematology analyzer, which uses spectrophotometry to quantify Hb concentrations [8]. This method is considered the gold standard and has minimal error due to the automation of laboratory processes, calibration and quality control checks [9,10]. In the field setting, particularly in large surveys and research studies where blood requires refrigerated transport over long distances, this method is usually not feasible.

Portable hemoglobinometers, such as the HemoCue® device, have become increasingly popular in the past decade in field settings and large surveys, as they are easy to use, inexpensive, portable, and provide an immediate digital Hb measurement [2]. This method is often used in nation-wide demographic and health surveys to determine the anemia prevalence of large populations [11].

Multiple studies have confirmed the accuracy and precision of HemoCue® compared to hematology analyzers to measure Hb in laboratory settings [12–15]. However, in resource-poor field settings, the HemoCue® device has shown bias and higher variability of Hb measures compared to hematology analyzers [16–21]. Other researchers have detected poor agreement and correlation between HemoCue® devices and hematology analyzers, notably among pregnant women in Sudan [22] and among pregnant women in Tibet living at high altitudes [23]. In these studies, researchers suggested that the HemoCue® method is not an acceptable method to use among the populations studied.

Abbreviations: CS, constant spring; Hb, hemoglobin; NIPHL, National Institute of Public Health Laboratory (Cambodia); WHO, World Health Organization.

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Issues relating to measurement error have been reported in the majority of studies; therefore, recommendations have been published to standardize blood collection and measurement practices [24–26]. There has been some investigation of the accuracy of HemoCue® to measure Hb in some clinical disease states, e.g. gastrointestinal bleeding [27] and as a screening tool for blood donations [28–30], however to our knowledge there has been no exploration of whether HemoCue® is as accurate as the gold standard hematology analyzer for measuring Hb in individuals with genetic Hb disorders.

In Cambodia, genetic Hb disorders affect ~50% of the population, the most prevalent of which are Hb E variants and alpha-thalassemia [31–33]. Hb E variants, in particular, result in an altered structure of the beta globin chain of Hb resulting in an unstable Hb molecule [34–36], hence the commonly used term 'structural variants'. These Hb E disorders are autosomal recessive, so can be inherited in either the heterozygous (also known as a 'trait') or homozygous form (which have more serious consequences) [37]. We speculated that if there were differences in measurement of Hb among individuals with Hb variants, it would be most apparent in the Hb E variants, given their high molecular instability.

2. Methods

2.1. Study design and participants

We evaluated two methods of measuring Hb concentration: a portable hemoglobinometer (HemoCue® Hb 201 + Hemocue AB) and an automated hematology analyzer (Sysmex XT-1800i). The study used data that were collected in July 2012 from 420 non-pregnant women in Prey Veng province in Cambodia as part of the baseline survey for a larger trial (unpublished). Women recruited were 18–45 y and had at least 1 child <5 y. The objective of the larger trial was to evaluate an improved model of homestead food production and aquaculture in rural Cambodia. Ethical approval for the study was granted by the Clinical Research Ethics Board at the University of British Columbia in Vancouver, Canada and the National Ethics Committee for Health Research in Phnom Penh, Cambodia.

2.2. Blood collection and analyses

A 3-h fasting capillary blood sample was taken from each woman (at her home) and processed using the HemoCue® device. Standard Hb 201 + microcuvettes were used to collect 2–3 drops of blood and were immediately inserted into the device for analysis. These specially designed microcuvettes act as a blood collection vessel and also contain sodium deoxycholate which disintegrates the erythrocyte membrane and releases Hb. Sodium nitrate then converts the Hb iron from ferrous to ferric state to form methemoglobin, which then combines with azide to form azidemethemoglobin. This compound is then measured by a spectrophotometer [38]. Phlebotomists were trained on procedures as per guidelines in the HemoCue® Hb 201 + operating manual [39] and standardized procedures [26].

The following morning, a 3-h fasting venous blood sample was collected from the same women at health centers in Prey Veng by trained phlebotomists from the Cambodian National Institute of Public Health Laboratory (NIPHL). Venous blood was collected in an evacuated 3.5 ml tube (Becton Dickinson) containing an anticoagulant (EDTA), placed on ice and transported daily to NIPHL in Phnom Penh for analysis. Venous blood was analyzed using a Sysmex hematology analyzer. A complete blood count was performed to determine Hb concentrations [40]. This system uses sodium lauryl sulfate to convert Hb to a colored compound that is measured by an automated spectrophotometer [8,41].

Quality control tests using both the HemoCue® device and Sysmex analyzer showed that both complied with minimum standards with the use of quality control solutions. Tests on the HemoCue® device were conducted using HemoTrol® (Level II) quality control solution (Eurotrol BV) at 3 different times and all control values were within acceptable levels ($\pm$6 g/l as defined by HemoTrol®). Quality control tests on the Sysmex analyzer were conducted by technicians at NIPHL using 3 different levels of Sysmex e-Check® control solution (Sysmex) and showed that all values were within acceptable limits (range of CV = 0.4–0.7%).

Genetic Hb disorders were identified using Hb electrophoresis and PCR [42]. A detailed methodology and the frequencies of identified genotypes in the 420 women have been published elsewhere [33]. Women were categorized into 3 groups based on the presence of Hb disorders and type of Hb disorders present: no Hb disorders (n = 195), Hb E variants (including heterozygosity or homozygosity with or without any other co-inherited variant, n = 133), and other Hb disorders (e.g. Hb Constant Spring (CS), α and β-thalassemia, n = 92). A minimum sample size of n = 50 is required for calculation of bias and precision in method comparison studies [43]. Therefore, we did not further segregate the Hb E variants into categories of heterozygosity and homozygosity due to the rarity of Hb E homozygotes and their corresponding small sample size (n = 31).

2.3. Data analyses and statistical methods

Hb concentration (g/l) is presented as the mean $\pm$ SD. The CVs were calculated to determine the relative variability of Hb concentration among each group of women. The prevalence of anemia in each group of women was determined using the Hb cut-off for non-pregnant women of reproductive age (Hb < 120 g/l) [2] and presented as the total number of women with anemia (n) and the proportion of women with anemia among the total study population (%).

The determination of bias (agreement) and precision (limits of agreement) were made [44]. Bias was defined as the difference in means between the 2 measures of Hb concentration (g/l) and was reported as the mean $\pm$ SEM. Precision was defined as the limits of agreement, or the 95% confidence intervals of the bias, and was reported as $\pm$ 1.96 SD. Precision plots are interpreted visually to compare discrepancies between methods (bias) and the width of the limits of agreement (precision and clinical significance), and any trends as the mean increases (consistency of variability).

Lin suggested the concordance correlation coefficient (ρ_c) as the most appropriate method to determine the reproducibility between two measured values, as it measures the departure of the measured values from a 45-degree line [45]. Reproducibility is also an important component of method-comparison studies, as when one or both methods do not provide repeatable results, then agreement is not useful to report alone [46]. Concordance was calculated and presented as the coefficient factor (ρ_c). In addition, concordance was plotted for 3 groups: women with no genetic Hb disorders, women with Hb E variants, and women with other Hb variants (not including Hb E variants). Pearson's correlation coefficient (r) was also reported for interest of comparison to the concordance coefficient. It is potentially misleading to solely use Pearson's coefficient in this comparison of two clinical measurements as it only measures the strength of the association and fails to detect agreement between values (departure from the 45° line) [44,45]. Accuracy in this study refers to the comparability of Hb measurements using the HemoCue® device and using a hematology analyzer, which is considered to be the gold standard method. If the bias is small and the limits of agreement are narrow (considering clinical significance) then it is suggested that the 2 methods are equivalent [44–46].

T-tests, ANOVA and chi-square tests were used to conduct pairwise comparisons and measure statistically significant differences between groups. Least significant difference (LSD) was used to adjust for multiple comparisons when required. Two-sided p-values less than 0.05 indicated statistical significance. Stata software version SE/13.1 for Mac (Stata Corp, College Station, Texas) was used to conduct statistical analyses.