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LETTER TO THE EDITOR

Comparison of hemoglobin concentrations measured by HemoCue and a hematology analyzer in Indian children and adolescents 1-19 years of age

Dear Editors,

Anemia is a severe public health problem among children and adolescents globally and in India, with potentially serious consequences for the health and development of children and physical and mental capacity of adolescents. The conventional automated hematology analyzer, based on the cyanmethemoglobin method, which is considered as gold standard method for Hb concentration measurement, though reliable and accurate, requires venous blood sample and adequate laboratory capacity, which limits its use in low-resource field settings, where portable handheld devices such as the HemoCue, which measures Hb in a capillary finger stick sample, are commonly used. As the HemoCue is less invasive and provides immediate results, it is widely used in field surveys. Accurate measurement of Hb concentration is critical for estimating anemia prevalence and formulating public health policy. The fourth National Family Health Survey (NFHS-4) conducted in India during 2015-16 reported an anemia prevalence of 59% among children under five years of age using hemocue.[1] In recent years, the precision and accuracy of field-based methods for Hb measurement have been questioned. Studies comparing Hb concentrations measured using the HemoCue against gold standard laboratory methods have shown mixed results in different age groups.

We came across only two studies[2,3] which compared hemoglobin (Hb) using hemocue in both capillary and venous blood with Hb in venous blood using autoanalyzer in young children. Therefore, in this study, we compared both capillary and venous blood Hb concentrations measured by HemoCue with Hb measured in venous blood on standard hematology analyzer and compared anemia prevalence estimates based on the three methods among children and adolescents 1-19 years of age in a survey setting in India. The study was conducted in the state of West Bengal and included participants of Comprehensive National Nutrition Survey (CNNS) conducted during 2016-18.[4]

Approval for the study was obtained from the ethics committee of the All India Institute of Medical Science (AIIMS). Informed consent was obtained from all caregivers of children aged 1-17 years and from adolescents aged 18-19 years.

Trained phlebotomists collected both capillary and venous blood samples within a gap of few minutes. Capillary blood collection followed standard procedures,[5] and Hb was assessed using HemoCue Hb 201$^+$ device (HemoCue AB). Venous blood samples were collected in vacutainers containing ethylenediaminetetraacetic acid (EDTA)-K3 (Becton Dickinson), and Hb was measured in hematology analyzer (LH 750/780, Beckman Coulter) as well as by HemoCue, for which the EDTA blood sample was mixed well by inversion 8-10 times and approximately 50 μL of whole blood was pipetted and placed on a sterile microscopic slide. A blood drop was aspirated into a standard microcuvette and immediately inserted into the HemoCue device. Rigorous quality control mechanisms were implemented for both the HemoCue and hematology analyzer.

Hb values for each method comparison (capillary HemoCue vs reference analyzer; venous HemoCue vs reference analyzer; capillary vs venous HemoCue) are presented as mean ± SD. Paired *t* tests were used to compare differences between mean Hb concentrations, and Pearson correlation coefficient (*r*) was calculated to assess the strength of association between Hb values measured using the three methods. A. The level of agreement for each comparison was assessed using Bland-Altman plots. The prevalence of anemia was determined based on WHO Hb cutoffs for children.[6] Reported *P* values are two-sided, and *P* values <.05 were considered to be statistically significant. Statistical analyses were conducted using STATA 15.0 (StataCorp).

A total of 754 children and adolescents aged 1-19 years were included in the study. Of these, 32% (n = 239) were 1-4 years, 38% (n = 285) were 5-9 years, and 30% (n = 230) were 10-19 years of age. Males and females comprised 51% and 49% of the study population.

Among the 754 children and adolescents, a capillary blood sample was collected from 623 (83%). Venous blood samples were collected from all 754 subjects. Hb concentration was measured in all venous samples using the hematology analyzer and in 579 (77%) using the HemoCue. Hb concentrations measured using all three methods were available for 484 (64%) participants.

Mean Hb concentration measured in venous blood by the hematology analyzer was 116 ± 12 g/L and that in venous blood and capillary blood by the HemoCue was 119 ± 12 g/L and 113 ± 15 g/L, respectively. There was a strong correlation between Hb concentration measured in venous blood by the HemoCue and hematology analyzer ($r = .9$; $P < .001$). A moderate correlation was observed between capillary blood HemoCue and in venous blood by the hematology analyzer ($r = .7$; $P < .001$). The positive and negative bias in venous and capillary HemoCue measurements, respectively, as compared to the reference analyzer, was also reflected in estimates

of anemia prevalence. Compared to the reference method, the venous HemoCue method underestimated anemia prevalence (43% vs 54%) and the capillary HemoCue method overestimated anemia prevalence (60% vs 54%).

The average biases for the venous HemoCue and capillary HemoCue were 3.0 ± 4.0 and −3.0 ± 11.0, respectively. In addition, the 95% limits of agreement around the mean were wider for capillary HemoCue (−23.6 to 18.1) than for venous HemoCue (−5.2 to 10.9). Subgroup analyses revealed weak correlation between capillary and venous HemoCue measures (r = .51) and between capillary HemoCue and venous analyzer measures (r = .58) among females. For males, both comparisons resulted in a moderate correlation (r = .74). A similar analysis by age group (1-4, 5-9, 10-19 years) revealed weak correlations in the 1-4 and 5-9 year age categories (data not shown).

Figure 1 shows the Bland-Altman plots of hemoglobin differences between values obtained in hematology analyzer and that in capillary blood by HemoCue, Figure 2 and Figure 3 are Bland-Altman plots for hemoglobin measured in venous blood by hematology analyzer and venous blood by HemoCue and capillary and venous HemoCue, respectively. The Bland-Altman plot revealed the maximum mean difference of 6.9 between capillary and venous HemoCue which reveals poor agreement between the two methods, while the limits of agreement (−22.74, 15.94) observed between reference hematology analyzer and venous hemocue indicate a good agreement.

In this study, a negative bias in Hb measured in capillary blood using the HemoCue was observed resulting in a 6% overestimation of anemia prevalence. In contrast, a positive bias was observed in Hb measured in venous blood using the HemoCue, which underestimated anemia prevalence by 11% compared to the hematology analyzer.

The strong correlation observed between Hb concentrations in venous blood measured using the HemoCue and hematology analyzer is consistent with previous studies that have shown a similarly high correlation. Munoz et al reported a strong correlation (r = .992; P < .01) between Hb in venous samples measured by the HemoCue B device and hematological analyzer Pentra 120 Retic (ABX).[7] A study conducted by Berry et al revealed a strong correlation (P < .0001) between fetal hemoglobin assessed by HemoCue and the hematology analyzer: Coulter S-Plus IV.[8]

There is mixed evidence from studies in children comparing Hb concentrations in capillary blood measured by HemoCue and in venous blood using a hematology analyzer. A review of 18 studies conducted in children aged 0-15 years revealed an underestimation of Hb concentration in capillary blood using the HemoCue in most studies, resulting in a 5%-15% overestimation of anemia prevalence.[9] However, some evidence suggests an overestimation of HemoCue measured Hb concentration in capillary blood.[10,11] In a recent study conducted among Laotian children 6-23 months of age, Hinnouho et al reported a significantly higher mean capillary Hb concentration using the HemoCue Hb 301, as compared to venous Hb concentration measured by a hematology analyzer.[12] In a study conducted among toddlers in the United States, Bougani et al reported a higher mean Hb concentration in capillary blood measured using the HemoCue, as compared to venous HemoCue and venous Coulter methods.[13]

In our study, Hb measurements differed according to the blood collection site, with higher venous vs capillary blood Hb concentrations measured using the HemoCue (capillary vs venous) than by analytic method (HemoCue vs hematology analyzer), where the limits of agreement between pairs of methods involving the HemoCue is broader (95% limits of agreement −1.962, 2.480) and dispersion of these differences are wider. Also, evidence suggests the sensitivity and specificity of HemoCue varies with the type of blood sample.[14,15]

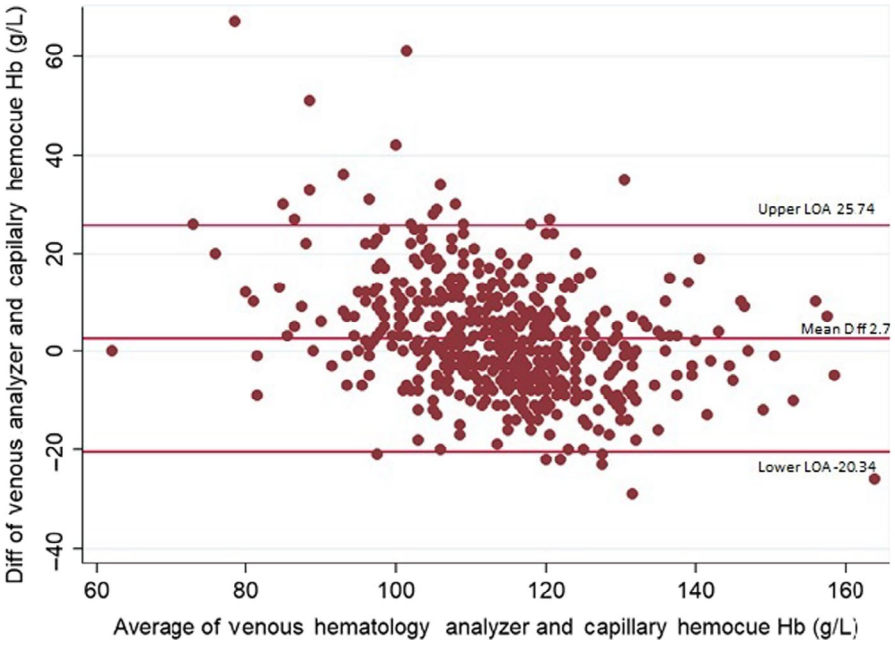

FIGURE 1 Bland-Altman plots were constructed to evaluate the agreement in Hb measurement between Hb values measured in venous blood by hematology analyzer and Hb values measured by hemocue in capillary blood, and the differences in Hb levels between the two techniques are graphically plotted against the averages of the two techniques. The mean difference (2.7) and upper (+22.74) and lower (−20.34) limits of agreement (LoA) are represented [Colour figure can be viewed at wileyonlinelibrary.com]

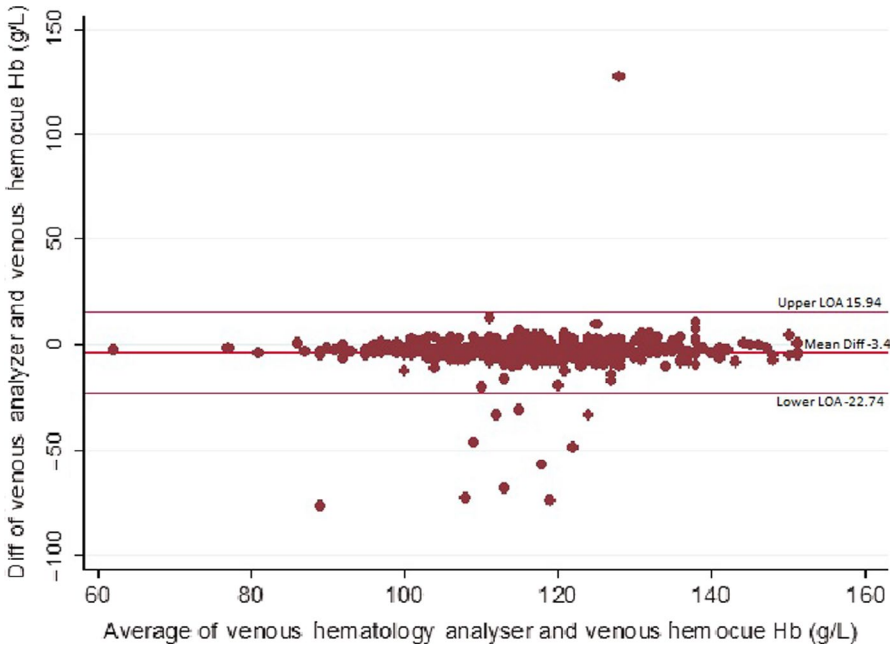

FIGURE 2 Bland-Altman plots were constructed to evaluate the agreement in Hb measurement between Hb values measured by hematology analyzer and hemocue in venous blood, and the differences in Hb levels between the two techniques are graphically plotted against the averages of the two techniques. The mean difference (−3.4) and upper (+15.94) and lower (−22.74) limits of agreement (LoA) are represented [Colour figure can be viewed at wileyonlinelibrary.com]

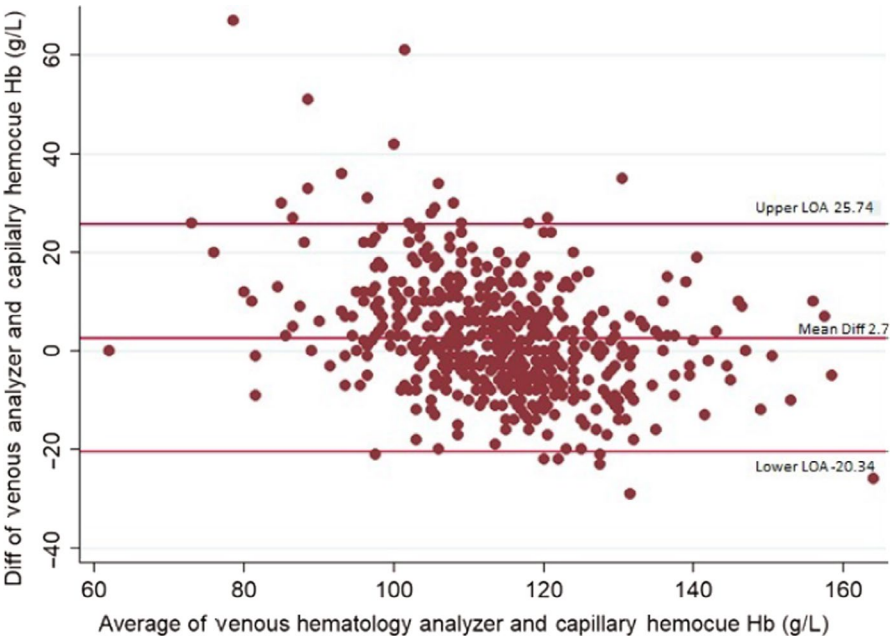

FIGURE 3 Bland-Altman plots were constructed to evaluate the agreement in Hb measurement between Hb values measured in venous blood and capillary blood hemocue, and the differences in Hb levels between the two techniques are graphically plotted against the averages of the two techniques. The mean difference (6.9) and upper (+35.28) and lower (−21.48) limits of agreement (LoA) are represented [Colour figure can be viewed at wileyonlinelibrary.com]

Several factors influence hemoglobin levels in capillary blood including skin temperature, depth of skin penetration, and dilution due to pressure exerted by milking, which may explain the difference in Hb concentrations observed between capillary and venous blood samples in our study.[16] However, the higher Hb concentration in venous blood measured by the HemoCue, as compared to the hematology analyzer, suggests differences in accuracy between the analytical methods, rather than actual differences in Hb concentration.

A strength of our study is that blood collection was carried out in a survey setting, and Hb was measured by the hematology analyzer which used the WHO-recommended cyanmethemoglobin method. A direct comparison of our findings with results from previous studies was not possible due to the various types of hemoglobinometers used in other studies (HemoCue Hb201, HemoCue B, HemoCue Hb301) and different biochemical methods for assessing Hb concentration.

The findings of our study warrant caution when interpreting hemoglobin levels measured using the HemoCue. Automated analyzers provide higher accuracy and precision, but their utility in survey settings currently is limited. Despite the greater costs and logistical challenges, hemoglobin measurement in field settings should be performed using more accurate methods to assess the burden of anemia.

KEYWORDS

anemia, hematology analyzer, HemoCue, hemoglobin, India

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest. The views expressed are those of the authors and do not necessarily reflect their respective organizations.

AUTHOR CONTRIBUTIONS

RAA contributed to design of work, acquisition and interpretation of data, and drafting the manuscript. PKA and RJ contributed to the conception of the work and revised the manuscript critically and provided intellectual content. Population Council team (SR, AK, AS, RA, NK) contributed to designing the work, acquisition, analysis and interpretation of data, and review of the manuscript. HPSS contributed to the conception of the work and revised the manuscript critically provided important intellectual content. UK and RS reviewed the manuscript and provided intellectual input. AJ reviewed the manuscript. AW reviewed the manuscript and provided intellectual input. SD and AK also reviewed the manuscript and provided intellectual input. LR contributed to the conception and design of the work, interpretation of data, drafting the manuscript and revised the manuscript critically, and provided intellectual content. All the authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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