Assessing the Validity of the Threefold Conversion between Hemoglobin and Hematocrit for the Determination of Anemia in Pregnancy

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Abstract –
Introduction: *The worldwide prevalence of anemia is higher among pregnant women. Anemic status is determined by the measurement of Hemoglobin (Hb) or Hematocrit (Hct). Hct (%) is usually defined as three times the value of Hb (g/dl). But the crude relationship between the two measures may be modified due to several factors such as; age, sex, season of survey and disease conditions. This study was therefore undertaken with the intent of assessing the validity of the 3- fold conversion between Hb and Hct to assess anemia in pregnant women.*

Method: *The Hb concentrations and Hct values from 70 pregnant mothers, aged 18-30 years, in their first trimester of pregnancy, from the ante-natal clinics of Teaching Hospital, Mahamodara were analyzed. The relationship between the concurrent measures of Hb and Hct was defined by linear regression analysis and the validity of the 3-fold conversion was assessed.*

Results: *The prevalence of anemia as defined by both Hb and Hct levels was 17%. Almost 59% of the microhematocrit values wrongly estimated Hb using 3- fold conversion. Sensitivity and specificity results obtained were below the reliability of clinical measurement. Regression models show that the association between the cut-offs of Hb and Hct is not dependent only on a simple conversion factor and correlation coefficient results are inadequate to conclude that there is a significant relationship between the two variables. A significant difference was observed between the values obtained using the two methods (t= 7.182, p <0.001).*

Conclusions: *The relationship between Hb and Hct is not exactly 3 in pregnant women and there is no simple conversion factor between the two measures. Since the Drabkin's method still has the advantage of being an international standard, this study argues for the consistent use of Hb rather than Hct in the assessment of anemia in pregnancy.*

Keywords – Hemoglobin, Hematocrit, Pregnancy, three-fold conversion

I. INTRODUCTION

A. *Anemia in pregnancy*

Anemia is a condition in which there is decreased level of Hb than normal or there is decreased number of RBC's than the normal value (Beutler & Waalen, 2006). It is a common and severe problem in many developing countries. Anemia in pregnancy is a special issue where, the normal physiological increase of both plasma volume and red cell mass with an excessive increase in plasma volume causes hemodilution in a pregnant woman resulting in a reduction of Hb levels approximately up to 11g/dl (Blackwell, 2008). According to the CDC (Centers for Disease Control and Prevention) criteria Hb cut-off used to define anemia during the first trimester of pregnancy is 11 g/dl and the corresponding cut-off for Hct is 0.33 (Gjorup, 1986).

Anemia in pregnancy is associated with deleterious effects such as fetal cerebral vasodilatation, increased risk of prematurity, spontaneous abortion, low birth weight, fetal death as well as increased risk of maternal mortality (Kalaivani, 2009). Pregnant women with anemia have a mortality rate three to five times higher than normal and a stillbirth rate six times higher than normal (Kalaivani, 2009). According to the United Nations estimates, approximately half of pregnant women suffer from anemia worldwide while the prevalence of anemia during pregnancy is 75% in South Asia **(**Karaoglu *et al.*, 2010; Brabin *et al*, 2001). Since early detection and effective management of anemia in pregnancy contributes substantially to the reduction in maternal mortality and stillbirth rate, it is crucial that all pregnant women are screened for anemia during pregnancy (Kalaivani, 2009; Den Broek *et al.*, 1999; Sherard & Newton, 2001).

B. Screening for anemia during pregnancy

Anemia is typically determined by measuring the Hb concentration in blood. In developed countries, where the prevalence of anemia is below 20%, an accepted standard practice is that all women have at least one measurement of Hb during the course of pregnancy (Brabin *et al.*, 2001). However, Packed Cell Volume (PCV) or Hct has been widely used as an alternative to Hb in various antenatal clinics and peripheral units.

C. *Hemoglobin and Hematocrit*

Both Hb and Hct refer to specific characteristics of red blood cells, however, they measure different factors. Hb provides a direct measure of the Oxygen carrying capacity of the blood, whereas Hct provides an indirect measurement of this. Both parameters can be assessed either with an automated blood counter or by manual methods such as microhematocrit method for Hct or colorimetric method for Hb (Sherard & Newton, 2001). Both Hct and Hb levels could be affected by factors such as the method and equipment used for its multivariate model: Hct = 3x Hb (De Benoist *et al.*, 1993).

Hb is considered to be superior to Hct for the purpose of monitoring anemia because of the availability of international reference standard preparations (Quinto *et al.*, 2006). In resource poor settings where automated hematology analyzers are not available, the Cyanmethemoglobin method is often used for Hb estimation. Nevertheless this method is time consuming and its disposal may create a problem due to large volumes of reagent which contains cyanide constitute a potential bio-toxic hazard (Kelleher *et al.,* 2001).

In situations where limited resources are available and the technical support is poor, a simple screening tool is likely to perform better than sophisticated methods that depend on correct dilutions and preparation of standards (Brabin *et al.*, 2001). Measurement of Hct using microhematocrit method is one such laboratory investigation which is simple to use, cheap and provides results within a very short period of time (5 min). As per the literature, microhematocrit method has an adequate level of accuracy and precision for clinical utility and therefore in many settings where automated methods for Hb determinations are not available, Hb values are estimated using observed Hct levels (Chakravarthy *et al.*, 2012). In general, in a rural setting, the running costs for Hct are very low and therefore in studies involving large populations it is cheaper to measure Hct (WHO, 2000). Further it is a less hazardous method that can be performed with less qualified personnel. Even a finger prick capillary blood sample is sufficient for performance of Hct testing by microhematocrit method.

D. Three-fold conversion between Hb and Hct

It is generally assumed that the conversion from Hb to Hct is pretty straightforward. Using the Hct value, there is a rough conversion factor of 3 which converts the Hct value to approximate Hb level. For example, if 33% of the blood contains red blood cells by volume, the Hb content would be about 33/3 = 11 g/dl (Jordan, 2009).

E. *Factors affecting the 3- fold conversion*

The crude relationship between Hb and Hct levels may be modified due to several factors. The literature has highlighted the fact that, it may vary with age, sex, season of survey and disease conditions such as malaria (De Benoist *et al.*, 1993). The relationship between Hct and Hb is expressed with the Mean Corpuscular Hemoglobin Concentration (MCHC). The MCHC varies depending on the type of anemia. An increased MCHC is seen in spherocytosis, whereas decreased levels may indicate iron deficiency, blood loss or thalassemia. It could be the case that obtaining a single conversion factor is not feasible, as the relationship depends on the prevalence of anemia in each population and on the type of anemia as well (De Benoist *et al.*, 1993).

The retrospective data of the studies done on the matter in different laboratories in different countries shows that the assessment of anemia using the 3- fold conversion between Hb and Hct has become a debatable issue with some studies showing positive correlation and some showing absence of any correlation (Carneiro & Drakeley, 2007**;** Lee *et al., 2008*; Rycel *et al.*, 2009). Therefore the potential for further improvements in the conversion factor certainly merits further investigation and analysis.

II. METHODS

A. Subjects and sampling

K_3- EDTA blood samples were collected by nursing officers in the course of routine Hb estimation in pregnant mothers (n=70) on their first visit to the ante-natal clinics (both hospital clinic and university; obstetrics and gynecology clinics) at Teaching Hospital Mahamodara. The first visit is usually made at 9 – 12 weeks gestation. The inclusion criteria included; subjects in the first trimester of pregnancy, aged between 18-30 and no history of chronic illness. Exclusion criteria included; age younger than 18 and older than 30, previous history of nutritional or hemolytic anemia and a history of hemoglobinopathies. Written consent was obtained from each participant and an interviewer administered questionnaire was filled in. The subjects were selected using non-probability purposive sampling technique. The minimum sample size required in purposive sampling is often fewer than 30. To increase the reliability of the results, considering the time and resources provided, sampling was continued up to 70. It was the size of sample affordable with the two months' time period of data collection. The study period was January 2013 – January 2014 (from literature review and proposal writing to dissemination of knowledge).

B. Laboratory methods

Blood samples were processed to measure Hb by Drabkin's method (cyanmethemoglobin method) using UV-1800, SHIMADZU recording double beam spectrophotometer (USA). Hct was assessed by centrifugation using Universal Microhematocrit Centrifuge (Hettich instrument, Germany) according to standard microhematocrit procedures (12000g, 5min).

C. Statistical analysis

All mothers with Hb values less than 11 g/dl were categorized into anemic group. Anemic pregnant mothers were further classified as severely anemic (Hb < 8 g/dl), moderately anemic (Hb 8 – 9.9g/dl) and mildly anemic (10 – 10.9g/dl (Gjorup*,* 1986).

Descriptive analysis was done first to explain the variation in the variables studied. Linear regression models were evaluated in order to evaluate the relationship between Hb and the Hct values. Sensitivity and specificity were calculated to assess the validity of the 3- fold conversion.

III. RESULTS

A. Prevalence of anemia among pregnant women

Approximately one sixth (17%) of the population had anemia during the first trimester of pregnancy evidenced by Hb concentrations of 8-11 g/dl. According to the results of both Drabkin's method and Hct method, 14.3% of the pregnant mothers were classified as having mild anemia and 2.9% as having moderate anemia (Table 1). About 83% pregnant mothers with an Hb concentration > 11 g/dl were classified as non-anemic.

Table 1. Distribution of anemia (n=70) in the study population

Subjects	Definition	Frequency, n (%)
Mild	Hb 10 – 10.9 g/dl	10 (14.3)
Moderate	Hb 8 – 9.9 g/dl	2 (2.9)
Severe	Hb < 8 g/dl	_
Total anemia	Hb < 11 g/dl	12 (17)

Approximately 50% out of the mild anemic cases which were classified as mildly anemic by Hb (Drabkin's method) were not considered anemic using the Hct level. For moderate anemia, the agreement was similar as 50% of the pregnant mothers classified as having moderate anemia by Hb did not classify as moderately anemic when using the Hct level. About 10% of subjects classified as mildly anemic by Drabkin's method were considered as moderately anemic by the Hct method (Table 2).

Table 2. Comparison of the results obtained by Drabkin's method and Microhematocrit method

	Hb values obtained by Drabkin's method (g/dl)						
Hb values by Hct/3 (g/dl)	8 – 9.9	10 – 10.9	11 – 11.9	12 – 12.9	13 – 13.9	≥14	Total
8 – 9.9	1	1	0	0	0	0	2
10 – 10.9	1	4	4	1	0	0	10
11 – 11.9	0	5	10	12	2	0	29
12 – 12.9	0	0	2	12	9	2	25
13 – 13.9	0	0	0	0	2	2	4
≥ 14	0	0	0	0	0	0	0
Total	2	10	16	25	13	4	70

	Hb values obtained by Drabkin's method (g/dl)						
Hb values by Hct/3 (g/dl)	8 – 9.9	10 – 10.9	11 – 11.9	12 – 12.9	13 – 13.9	≥14	Total
Correctly estimated by Hct/3	1/2 (50%)	4/10 (40%)	10/16 (62.5%)	12/25 (48%)	2/13 (15.4%)	0/4 (0%)	

B. Drabkin's method Vs. 3-fold conversion

A comparison of different variables for Drabkin's method and 3- fold conversion by microhematocrit method is given in table 3.

Table 3. Comparison of Hct/3 measurements and Drabkin's method for Hb estimation

	Hct / 3 Measurements	Drabkin's method
No: of samples	70	70
Mean Hb (g/dl)	11.6	12.2
Median Hb (g/dl)	11.7	12.2
SD	0.81	1.14
Lowest Hb (g/dl)	9.70	9.40
Highest Hb (g/dl)	13.3	15.1
Range	3.60	5.70
Student t- test	7.182	P< 0.001

C. Validity of the 3- fold conversion between Hb and Hct

The sensitivity and specificity results of the 3- fold conversion considering the whole population were 58.3% and 91.4% respectively. The validity of the conversion factor at different cut-off points of Hb concentrations considering the Drabkin's method as the Gold Standard is shown in Table 4.

Table 4. Validity of the 3- fold conversion between Hb and Hct at different cut-off points of Hb concentrations

Results	Hemoglobin concentrations (g/dl)					
	8 – 9.9	10 – 10.9	11 – 11.9	12 – 12.9	13 – 13.9	≥ 14
True positive	1	4	10	12	2	0
True negative	1	4	10	12	2	0
False positive	1	6	19	13	2	0
False negative	1	6	6	13	11	4
Sensitivity (%)	50	40	62.5	48	15.4	0
Specificity (%)	50	40	34.5	48	50	0

D. Correlation

Figure 1, 2 and 3 shows the relationship between **Hb and Hct levels for the study samples, using** different regression models. A positive correlation was observed between the results of the two methods with a correlation coefficient (r) of 0.799 (r^2 = 0.637) considering the whole study population. Though the results showed good correlations there were some random differences in readings, especially at higher levels of Hb concentrations. The intercept (a = 13.91) and slope (b = 1.711) suggest that there is a bias in the results (ideally a = 0 and b = 3) and there is no simple conversion factor between the two measures as the relationship is: **Hct = (1.711x Hb) + 13.91** (Figure 1).

At mild anemia, the relationship between Hb and Hct as expressed by the regression line was: **Hct = (2.601xHb) + 4.621** with a 0.559 of Correlation Coefficient (r) (Figure 2). The number of subjects with moderate anemia (2 mothers) was inadequate to conclude with a relationship between the two values.

To avoid the possibility of bias, separate regressions were performed for the determination of the conversion factor for non-anemic pregnant women (Figure 3).The equation derived through linear regression for non-anemic pregnant mothers was **Hct =(1.644x Hb) + 14.78** with a strong positive correlation (r=0.715) between the two variables.

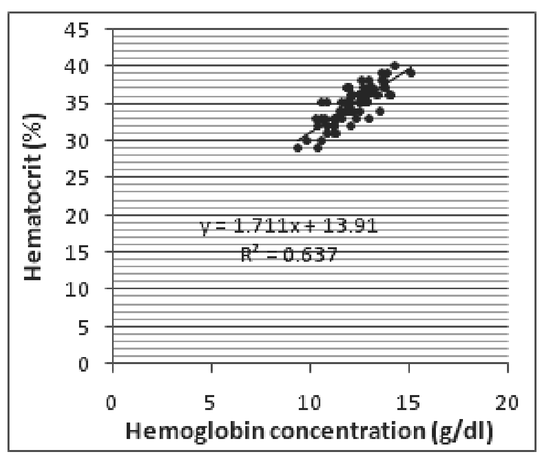

Figure 1. Scatter- plots of Hct values against Hb measurements

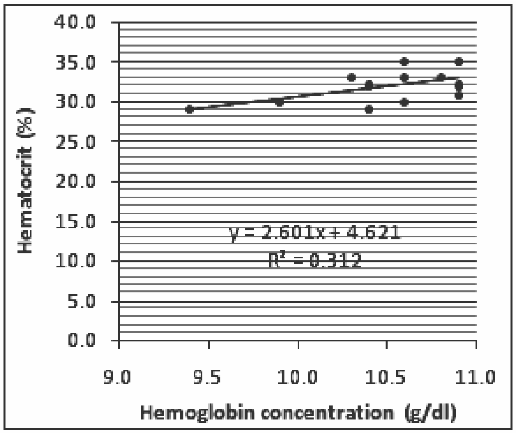

Figure 2. Relationship between Hb and Hct at mild anemia

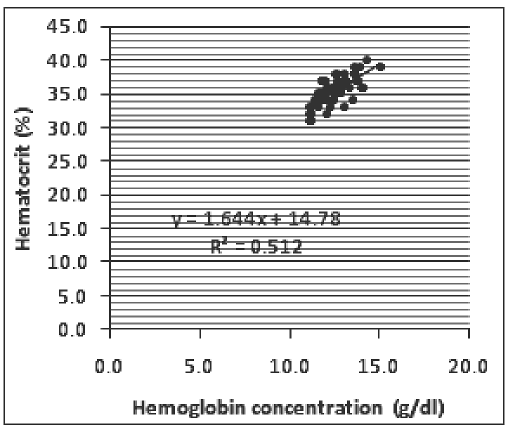

Figure 3. Relationship between manual Hb and Hct at Hb concentrations ≥11 g/dl

IV. DISCUSSION

Anemia in pregnancy constitutes a real concern all over the world including developing countries like Sri Lanka. A research done in 2001 revealed that the prevalence of anemia among Sri Lankan pregnant women was 29.3% (WHO, 2001). Findings of the present study (prevalence of 17%) were not close to those figures and the reduction in anemia prevalence is expectable due to the socio-economic growth of Sri Lankan population over the past few years since governments conducted programs related to mother and child health such as antenatal care, nutrition education of the public etc.

As indicated previously, anemia may have an adverse impact on maternal and fetal well-being and pregnancy outcome. Significant effort is therefore given to monitoring and responding to hematological parameters (Bland & Altman, 1986). The gold standard laboratory investigation for the detection of anemia is measurement of Hb concentration using Cyanmethemoglobin (Drabkin's) method. The conversion factor of 3 which converts the Hct value to approximate Hb level was considered in this study.

The mean Hb value obtained from Drabkin's method was 12.2 g/dl while the corresponding value derived from 3- fold conversion of Hct was 11.6 g/dl. The lower mean value suggests that, the 3- fold conversion would underestimate the Hb values. The range of Hb results by Drabkin's method was more elaborate (5.7 g/dl) while the results derived by Hct method was more compact (3.6 g/dl). This suggests that Drabkin's method is more sensitive and has an obvious advantage over the 3-fold conversion which is less sensitive.

The results showed a consistent bias between the two measures, with Hb concentrations by Drabkin's method being higher than the values derived from 3- fold conversion in 81% of the observations. There was a significant difference between the two measures especially at higher Hb concentrations and this was confirmed by the student t-test ($p<0.001$). The detection of anemia was correctly made in 50% and 40% of moderate and mild anemic cases respectively by Hct method. About 10% of subjects classified as mildly anemic by Drabkin's method were considered as moderately anemic by the 3- fold conversion. These variations in Hb results by the two methods would compromise the validity of the 3- fold conversion.

For the purpose of screening an antenatal population for anemia, high sensitivity is desirable since it is important that as many individuals as possible with anemia have a positive test result. But the sensitivity results obtained from this study was below the reliability of clinical measurement for Hct conversion method. With the increase in Hb concentrations the sensitivity results seemed to be decreasing while the specificity results varied.

Previous studies have used correlation to compare two measurement methods (Bland & Altman, 1986). Regression models show that the association between the cut-offs of Hb and Hct was not dependent only on a simple conversion factor (Figure 1). Though a positive correlation ($r = 0.799$)

was observed when considering Hb and Hct values, the line of best fit indicates that only 64% (r^2 = 0.637) of the variation in Hct values is explained by the regression line. 36% of the variation may be due to other factors which are not captured in our regression model. As mentioned in the literature, the factors such as age, seasonal variations and exposure to disease conditions such as malaria may have modified the crude relationship between Hb and Hct (Bland & Altman, 1986).

When considering the non-anemic pregnant women, the correlation between the two variables was a strong positive correlation with a Correlation Coefficient of 0.715 (Figure 3). The Correlation Coefficient for mild anemia (Figure 2) was 0.559 (r^2 = 0.312) and those results were comparable to the research by Lee SJ *et al* (**2008).** However, as suggested by Cornbleet & Gochman (1979), in any analysis if the data is collected over a narrow range, the estimate of the regression parameters is relatively imprecise and may be biased. The correlation coefficient can be used as a guide to assess the adequacy of the comparative method range in overcoming this problem and the range of data can be considered adequate if $r > 0.975$. Considering the narrow range involved in this study, correlation coefficient results are inadequate to conclude that there is a significant relationship between the two variables.

It was possible to derive comparable Hb levels from Hct method using different equations at different cut-off concentrations in this study (see Figure 1-3). The requirement of different conversion factors would compromise the reliability of practical use of Hct in determination of Hb results. Therefore the standard 3-fold conversion between the two measures cannot be considered as valid for the assessment of anemia.

The reasons for these different conversion factors were not analyzed during this study but it is suspected that physiological changes during pregnancy may have an effect on it. Errors of microhematocrit procedure such as sampling errors (prolong stasis, inadequate mixing with anticoagulants), errors in filling or sealing, reading errors and packing errors during the microhematocrit procedure may also have given rise to the difference between the results of two methods. Also there is a possibility of environmental factors and subject's differences affecting the results due to the long interval between sampling and performing the test. There can be time dependent shrinkage of red cells which can contribute to Hct results. However the anticoagulant used was K_3-EDTA which has shown least influence on analyze on storage (CLSI guidelines).

V. CONCLUSIONS

These data show that Hb levels cannot be derived from the Hct values with an acceptable accuracy using the general rule of dividing by 3. The conversion factor between Hb and Hct is not exactly 3 in pregnant women and there is no simple conversion factor between the two measures. Since the Drabkin's method still has the advantage of being an international standard, this study argues for the consistent use of Hb rather than Hct in the assessment of anemia in pregnancy.

VI. LIMITATIONS

Exclusion of pregnant mothers from other antenatal clinics (MOH clinics) and hospitals may limit the specificity of this investigation. Not performing serum folate level, peripheral blood film microscopy and serum iron profile (for the confirmation and differentiation of anemia) due to financial constraints was another shortcoming. ROC curve to assess validity of the 3-fold conversion was not applicable due to small sample size.

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