Maternal anaemia and its effects on neonatal anthropometric parameters in patients attending a tertiary care institute of Solan, Himachal Pradesh, India

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ABSTRACT

Background: Anaemia during pregnancy is a significant concern in India. The consequences of iron deficiency anaemia during pregnancy are often serious and long lasting for both the mother and the foetus. Haematocrit measurement is an acceptable and recommended method for anaemia determination especially in situations where limited resources are available, and the technical support is poor. There is a rough conversion factor of 3 which converts the HCT value to approximate haemoglobin level. Present study desires to know the prevalence of anaemia (with classification into mild, moderate and severe) in 3rd trimester pregnancy, to know effect of anaemia in pregnancy on new-born anthropometric parameters and to assess the validity of the threefold conversion between haemoglobin and haematocrit for the determination of anaemia in pregnancy.

Methods: The estimation of haematocrit was done by micro-haematocrit method and estimation of haemoglobin was done by automated blood cell analyzer based on cyanmethemoglobin method. Welch's ANOVA, Post Hoc games Howell test and Bland Altman limits of agreement method were used for statistical analysis.

Results: Present study showed that 53.75% women in their 3rd trimester were anaemic (mild, moderate and severe anaemic mothers were 22.25%, 28.25% and 3.25% respectively). The standard 3-fold conversion between the haemoglobin and haematocrit was not found to be valid for the assessment of anaemia in the 3rd trimester pregnancy. Finally, while comparing anthropometric measurements between mild/no anaemia group with severe anaemia group we found that all measurements were less in severe anaemia group and this difference was statistically significant.

Conclusions: This study shows that 53.75% women in their 3rd trimester were anaemic, which closely mimic the WHO data but is about 11% more than the prevalence in Himachal Pradesh. Secondly, the standard 3-fold conversion between the haemoglobin and haematocrit was not found to be valid for the assessment of anaemia in the 3rd trimester pregnancy. Finally, birth weight, height, head circumference, chest circumference and mid-arm circumference were significantly affected by third trimester haemoglobin that too the most in severe anaemia cases.

Keywords: Anaemia, Pregnancy, Hematocrit, Hemoglobin

INTRODUCTION

Anaemia during pregnancy is a significant concern with the World Health Organization (WHO) estimating a prevalence of 54%, 39.3% and 38.2% amongst pregnant women in India, Asia and world respectively.¹ In healthy, iron-sufficient women, haemoglobin (Hb) concentrations change dramatically during pregnancy to accommodate the increasing maternal blood volume and the iron needs of the fetus.² Currently, there are no WHO
recommendations on the use of different haemoglobin cut-off points for anaemia by trimester, but it is recognized that during the second trimester of pregnancy, haemoglobin concentrations diminish appreciate 0.5 gm/dl.\(^3\) The WHO defines anaemia regardless of its cause as the presence of a Hb level of less than 11.0 gm/dl during pregnancy.\(^3,4\) Mild anaemia is defined as Hb between 10.0-10.9 gm/dl, moderate anaemia as 7.0-9.9 gm/dl and severe anaemia as <7 gm/dl.\(^3,5\) The US Centres of Disease Control (CDC) recommendations take into account the observation that there is a trough in the physiological course of Hb during pregnancy.\(^6\) According to this definition, anaemia is present if the Hb level is less than 11 gm/dl during first trimester and third trimester of gestation, and less than 10.5 gm/dl during second trimester. These Hb levels correspond to haematocrit (Hct) values of 33.0, 32.0 and 33.0\%, respectively.\(^5,7\) The consequences of iron deficiency anaemia (IDA) during pregnancy are often serious and long lasting for both the mother and foetus. Expectant mothers with anaemia often experience increased fatigue levels, reduced exercise performance and reduced mental performance.\(^8,9,11\) Furthermore, severe anaemia is related to an increased risk of spontaneous abortions, prematurity, small for gestational-age babies, intrauterine growth retardation, and neonatal death.\(^8,12,14\) Since early detection and effective management of anaemia in pregnancy contributes substantially to the reduction in maternal mortality and stillbirth rate, it is crucial that all pregnant women are screened for anaemia during pregnancy.

**Haemoglobin and haematocrit**

The Hb provides a direct measure of the oxygen carrying capacity of the blood, whereas the Hct provides an indirect one. The Hb estimates the erythrocytic function and is more stable to plasma volume changes such as dehydration and RBC shape, which makes it somehow more reliable. Both the method of haemoglobin measurement and blood sample source can affect the measured haemoglobin concentration. The cyanmethemoglobin and the HemoCue system are the methods generally recommended for use in surveys to determine the population prevalence of anaemia.\(^15,16\) The cyanmethemoglobin measurement is the reference laboratory method for the quantitative determination of haemoglobin and is used for comparison and standardization of other methods.\(^15,16\) The HemoCue system is based on the cyanmethemoglobin method and has been shown to be stable and durable in field settings.\(^16,17\) Haematocrit or packed cell volume is a commonly performed clinical assessment frequently used in surveys of anaemia because of its simplicity and the widespread availability of the necessary equipment. Hb is considered to be superior to Hct for the purpose of monitoring anaemia as it has an internationally accepted reference standard calibrator for cyanmethemoglobin method.\(^18,19\) Automated cell counters now also use Sodium lauryl sulphate instead of toxic reagents (cyanmethemoglobin method).\(^20,21\) In resource poor settings where automated haematology analysers are not available, the manual 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.\(^20,21\) 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. As per the literature, microhaematocrit 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. 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.\(^22\) Further it is a less hazardous method that can be performed by less qualified personnel.

**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.\(^4,23,24\) Hb and Hct and 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.\(^18,19,25-30\) It could be the case that obtaining a single conversion factor is not feasible, as the relationship depends on the prevalence of anaemia in each population and on the type of anaemia pre-dominating within it. Various studies have been done on this matter in various countries and they show that the assessment of anaemia 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.\(^19,27-29,31\)

Only one study has been done regarding validity of this conversion in pregnant females which shows poor correlation of this conversion factor.\(^30\) Therefore, before commenting on reliability of this conversion factor and the potential for further improvements in the conversion factor certainly merits further investigation and analysis in pregnancy.

The objectives of the present study were:

- To assess the validity of the threefold conversion formula between haemoglobin and haematocrit for the determination of anaemia in 3rd trimester of pregnancy.
- To know the prevalence of anaemia with its grading into mild, moderate and severe in pregnancy (3rd trimester) in area of Himachal Pradesh around Maharishi Markandeshwar Medical College and Hospital, Solan, Himachal Pradesh, India.
• To know whether mild, moderate or severe anaemia in 3rd trimester pregnancy is associated with less birth weight, head circumference, chest circumference, mid-arm circumference and/or length of the newborn.

METHODS

This prospective cross-sectional study was done w.e.f March 2017, in Maharishi Markandeshwar Medical College and Hospital, Solan, Himachal Pradesh, India. Four hundred pregnant women who gave birth at the Obstetrics department and their newborns were included in this study.

Inclusion criteria

All singleton pregnancies with gestational age of more than 37 completed weeks, admitted in the 3rd trimester for the delivery of baby in Obstetrics and Gynaecology Department of Maharishi Markandeshwar Hospital and Medical College, Solan, Himachal Pradesh.

Exclusion criteria

• Multiple pregnancies
• Gravidae 4 or more, pregnancy in 1st or 2nd trimester
• Positive history of smoking cigarettes, tobacco use, alcohol or narcotic use
• Age less than 18 years and more than 40 years
• History of any chronic illness, other diagnosed causes of non-nutritional anaemia
• Liver, kidney and/or heart disease, diabetes, hypertension, severe sepsis
• History of menorrhagia, any visible blood loss or blood transfusion in last 12 months
• Babies born with congenital malformations

Procedure

A written and informed consent was obtained from each participant/family members and an interviewer administered questionnaire was filled before starting the procedure/study. Venous blood samples of the cases were collected under minimal stasis in Sodium EDTA (ethylene diamine tetra acetic acid of 2 mg/ml concentration) containing glass tubes. These tubes were adequately labelled and immediately sent to laboratory. These samples were stored at 4°C and processed within 6 hours of taking the sample.

The estimation of hematocrit was done by microhematocrit method, as recommended by WHO.20 Reporting was done in percentage units. In each case this hematocrit value was divided by 3 to get a value which was denoted as calculated hemoglobin and its reporting was done in grams/decilitre (gm/dl). Estimation of hemoglobin was done by automated blood cell analyzer based on cyanmethemoglobin method from the same samples. The values were denoted as measured hemoglobin and reporting was done in gm/dl.

Anaemia was defined and classified according to WHO guidelines as the presence of an Hb level of less than 11.0 gm/dl during pregnancy.3-5 All the anthropometry parameters of neonates were done at 24-48 hours of life and were taken as per guidelines given by WHO.32

Statistical methods

IBM SPSS statistics software and Analyse-it statistics software for Microsoft excel 4.91.3 was used for the study. Continuous variables were expressed as standard descriptive statistical calculations (mean, median, mode, standard deviation etc.). An assessment of the normality of data, wherever required was done by Shapiro-Wilk test. Welch's ANOVA test, Post Hoc Games Howell multiple comparison test and Bland Altman limits of agreement method was used in this study. In Bland Altman method, bias and precision estimates of ±1 gm/dl and ±1 gm/dl, respectively, were established a priori as the maximum parameters that would indicate acceptable agreement between methods and precision of the difference.33,34

RESULTS

This study included a total of 400 subjects and their respective newborns. Minimum and maximum maternal age was 19 years and 38 years respectively. Mean and median age was 25.2 years and 25 years respectively with standard deviation of 3.5 years.

Table 1: Categorization of cases according to measured haemoglobin levels.

<table>
<thead>
<tr>
<th>Variable groups</th>
<th>Number of cases (%)</th>
<th>Minimum haemoglobin (gm/dl)</th>
<th>Maximum Haemoglobin (gm/dl)</th>
<th>Median (gm/dl)</th>
<th>Mode (gm/dl)</th>
<th>Mean (gm/dl)</th>
<th>Standard Deviation (gm/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaemic</td>
<td>215 (53.75)</td>
<td>05.00</td>
<td>10.90</td>
<td>09.80</td>
<td>10.30</td>
<td>09.43</td>
<td>01.26</td>
</tr>
<tr>
<td>Non-anaemic</td>
<td>185 (46.25)</td>
<td>11.00</td>
<td>14.60</td>
<td>12.00</td>
<td>11.40</td>
<td>12.13</td>
<td>00.88</td>
</tr>
<tr>
<td>Mild anaemia</td>
<td>89 (22.25)</td>
<td>10.00</td>
<td>10.90</td>
<td>10.50</td>
<td>10.30</td>
<td>10.47</td>
<td>00.29</td>
</tr>
<tr>
<td>Moderate anaemia</td>
<td>113 (28.25)</td>
<td>07.10</td>
<td>09.90</td>
<td>09.20</td>
<td>09.90</td>
<td>08.96</td>
<td>00.85</td>
</tr>
<tr>
<td>Severe anaemia</td>
<td>13 (3.25)</td>
<td>05.00</td>
<td>06.90</td>
<td>06.60</td>
<td>06.90</td>
<td>06.34</td>
<td>00.67</td>
</tr>
<tr>
<td>Total cases</td>
<td>400 (100)</td>
<td>05.00</td>
<td>14.60</td>
<td>10.80</td>
<td>11.40</td>
<td>10.68</td>
<td>01.74</td>
</tr>
</tbody>
</table>
Out of total of 400 cases, 209 (52.25%) were normal vaginal deliveries, 64 (16%) were elective lower segment caesarean section (LSCS) cases, 103 (25.75%) were emergency LSCS cases, 1 (0.25%) was by forceps assisted vaginal delivery and 23 (5.75%) were vacuum assisted vaginal deliveries. LSCS constituted 167 cases i.e. 41.75% of the total. Out of a total of 400 new-borns, 175 new-borns (43.75%) were male and the rest females. 215 maternal cases were anaemic among which 41.39% were mildly anaemic, 52.56% were moderately anaemic and 6.05% were severely anaemic. Detailed characteristics of the subjects and new-borns are as outlined in Table 1 and 2.

**Comparison between measured haemoglobin and calculated haemoglobin (all groups)**

Comparison of means by paired (dependent) sample T test showed significant difference between the measured Hb and calculated Hb (p=0.005).

A scatter diagram of the measured haemoglobin and calculated haemoglobin was made. Most of Hb values are seen closely clustered around and also both distributed above and below the line of equality in a linear fashion, though a few outliers are also seen. Pearson's correlation coefficient (r) was 0.938, with a significance level of p <0.0001, and 95% confidence interval (CI) for r of 0.925 to 0.948.

The correlation analysis results tell that: the calculated Hb is very highly and positively associated with the measured Hb; the probability that this association was due to chance is less than 1 in 10,000; and when these methods are used in similar conditions, we can be confident that the r will be between 0.925 and 0.948. However, the strong correlation does not tell us about agreement between the methods. Bland Altman method was used for this purpose.

### Table 2: Anthropometric parameters of new-borns.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of cases (n)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Median</th>
<th>Mode</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>400</td>
<td>14.00</td>
<td>55.50</td>
<td>49.10</td>
<td>50.00</td>
<td>49.15</td>
<td>02.47</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>400</td>
<td>30.50</td>
<td>39.00</td>
<td>34.20</td>
<td>34.00</td>
<td>34.19</td>
<td>01.30</td>
</tr>
<tr>
<td>Chest circumference (cm)</td>
<td>400</td>
<td>27.50</td>
<td>36.80</td>
<td>32.10</td>
<td>31.20</td>
<td>32.09</td>
<td>01.44</td>
</tr>
<tr>
<td>Birth weight (gm)</td>
<td>400</td>
<td>1770.00</td>
<td>4055.00</td>
<td>2772.50</td>
<td>2800.00</td>
<td>2781.20</td>
<td>0401.10</td>
</tr>
<tr>
<td>Mid-arm circumference (cm)</td>
<td>400</td>
<td>07.90</td>
<td>14.00</td>
<td>10.20</td>
<td>10.50</td>
<td>10.34</td>
<td>01.12</td>
</tr>
</tbody>
</table>

While interpreting Bland Altman plot, the bias for each paired measurement point varied (bias is both positive and negative), across all paired measurements the average difference was 0.091 gm/dl, the value that would be reported as the bias for this data set. This bias is less than the priori criteria of 1 gm/dl. The confidence limit is 2.529 gm/dl which is more than a priori criterion of 1 gm/dl.

So, one can conclude that though the accuracy of the test method is acceptable, but repeatability is not acceptable. It is also noted that 21 data points exceed the limits of agreement; 10 exceed the upper limit and 11 exceeds the lower limit. Percentage error is 23.68% which means that haemoglobin differences between two methods depend upon the haemoglobin value i.e. severity of anaemia.

### Table 3: Descriptive comparison of measured and calculated Hb values.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Range</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured Hb*</td>
<td>400</td>
<td>9.60</td>
<td>5.00</td>
<td>14.60</td>
<td>10.68</td>
<td>1.74</td>
</tr>
<tr>
<td>Calculated Hb*</td>
<td>400</td>
<td>12.20</td>
<td>3.67</td>
<td>15.87</td>
<td>10.77</td>
<td>1.85</td>
</tr>
<tr>
<td>Valid N (list wise)</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Hb is in gm/dl

**Comparison between measured and calculated haemoglobin (no anaemia group)**

Pearson's correlation coefficient (r) was 0.808, with a significance level of p <0.0001, and 95% confidence interval (CI) for r of 0.751 to 0.853. The bias was 0.089 gm/dl which is less than the priori criteria of 1 gm/dl. The confidence limit is 2.75 gm/dl which is more than a priori criterion of 1 gm/dl. Percentage error is 22.67%. So, one can conclude that though the accuracy of the test method is acceptable, but repeatability is not acceptable.
Comparison between measured and calculated haemoglobin (Mild anaemia group)

Pearson's correlation coefficient (r) was 0.215, with a significance level of p <0.0001, and 95% confidence interval (CI) for r of 0.007 to 0.405. The bias was 0.085 gm/dl which is less than the priori criteria of 1 gm/dl.

Table 4: Bland Altman analysis of the measured and calculated haemoglobin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>95% CI</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean difference</td>
<td>0.091</td>
<td>0.0274 to 0.1542</td>
<td>0.0323</td>
</tr>
<tr>
<td>95% lower LoA</td>
<td>-1.174</td>
<td>-1.2822 to -1.0652</td>
<td>0.0552</td>
</tr>
<tr>
<td>95% upper LoA</td>
<td>1.355</td>
<td>1.2468 to 1.4637</td>
<td>0.0552</td>
</tr>
<tr>
<td>SD</td>
<td>0.645</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI: Confidence interval; SD: Standard deviation; SE: Standard error; LoA: Limits of agreement; *Hb is in gm/dl

The confidence limit is 1.82 gm/dl which is more than a priori criterion of 1 gm/dl. Percentage error is 17.38%. So, one can conclude that though the accuracy of the test method is acceptable, but repeatability is not acceptable.

Comparison between measured and calculated haemoglobin (Moderate anaemia group)

Pearson's correlation coefficient (r) was 0.784, with a significance level of p <0.0001, and 95% confidence interval (CI) for r of 0.700 to 0.846. The bias was 0.086 gm/dl which is less than the priori criteria of 1 gm/dl. The confidence limit is 2.49 gm/dl which is more than a priori criterion of 1 gm/dl. Percentage error is 27.79%. So, one can conclude that though the accuracy of the test method is acceptable, but repeatability is not acceptable.

Comparison between measured and calculated haemoglobin (Severe anaemia group)

Pearson’s correlation coefficient (r) was 0.667, with a significance level of p <0.0001, and 95% confidence interval (CI) for r of 0.183 to 0.891. The bias was 0.195 gm/dl which is less than the priori criteria of 1 gm/dl. The confidence limit is 3.835 gm/dl which is more than a priori criterion of 1 gm/dl. Percentage error is 60.49%. So, one can conclude that though the accuracy of the test method is acceptable, but repeatability is not acceptable. Association of severity of anaemia in 3rd trimester pregnancy with birth weight, head circumference, chest circumference, mid-arm circumference and/or length of the new-born.

Table 5: Correlation between measured Hb and various anthropometry parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chest circumference (cm)</th>
<th>Mid-arm circumference (cm)</th>
<th>Head circumference (cm)</th>
<th>Length (cm)</th>
<th>Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured Hb*</td>
<td>0.478</td>
<td>0.464</td>
<td>0.471</td>
<td>0.473</td>
<td>0.551</td>
</tr>
<tr>
<td>Measured Hb*</td>
<td>0.466</td>
<td>0.460</td>
<td>0.465</td>
<td>0.443</td>
<td>0.554</td>
</tr>
<tr>
<td>Measured Hb*</td>
<td>0.330</td>
<td>0.333</td>
<td>0.334</td>
<td>0.315</td>
<td>0.407</td>
</tr>
</tbody>
</table>

*Hb is in gm/dl

By seeing these parameters, we can say that weight and length visibly decrease as the severity of anaemia increases. HC, CC and MAC also show decrease especially in moderate and severe anaemia groups. As assumption of homogeneity of variances was violated, Welch’s ANOVA (Analysis of variance between groups) test was used in the statistical comparisons of groups and Post Hoc Games Howell multiple comparison test was utilized in the comparison of subgroups. p value of less than 0.05 and 0.01 was treated as significant in Welch’s ANOVA test and Post Hoc Games Howell multiple comparison test respectively.

Table 6: Neonatal anthropometric parameters in no anaemia and various anaemia groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No anaemia Group</th>
<th>Mild anaemia Group</th>
<th>Moderate anaemia group</th>
<th>Severe anaemia group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight* Mean (SD)</td>
<td>2956.49 (369.15)</td>
<td>2803.43 (282.685)</td>
<td>2536.50 (368.726)</td>
<td>2261.92 (268.022)</td>
</tr>
<tr>
<td>Length* Mean (SD)</td>
<td>50.1373 (2.09125)</td>
<td>49.0596 (2.08641)</td>
<td>48.0027 (2.49752)</td>
<td>45.6000 (2.05629)</td>
</tr>
<tr>
<td>Head circumference* Mean (SD)</td>
<td>34.6773 (1.23391)</td>
<td>34.2079 (1.12938)</td>
<td>33.5531 (1.15380)</td>
<td>32.5923 (0.74326)</td>
</tr>
<tr>
<td>Chest circumference* Mean (SD)</td>
<td>32.6897 (1.27067)</td>
<td>31.9551 (1.24024)</td>
<td>31.4850 (1.36364)</td>
<td>29.9308 (1.04034)</td>
</tr>
<tr>
<td>Mid-Arm circumference* Mean (SD)</td>
<td>10.7432 (1.06830)</td>
<td>10.4101 (0.81937)</td>
<td>9.7779 (1.04631)</td>
<td>8.9077 (1.05551)</td>
</tr>
</tbody>
</table>

*Weight is in gram. Length, Head circumference, chest circumference, mid-arm circumference is given in centimetre
Anthropometry parameters and Anaemia status comparison

The Welch ANOVA analysis tells us that the differences between increasing Hb concentrations in 3rd trimester pregnancy and all anthropometric parameters of newborns are statistically significant. Using the same data and same 4 groups (no anaemia, mild, moderate and severe anaemia) Welch ANOVA was further developed with a post hoc Games Howell test. While taking weight as dependent variable, relationship between weight and Hb was not statistically significant between moderate anaemia and severe anaemia group (p=0.017). Rest of combinations were statistically significant.

Table 7: Welch ANOVA test with weight (gm) as dependent variable.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welch</td>
<td>45.763</td>
<td>3</td>
<td>57.235</td>
</tr>
<tr>
<td>Brown-Forsythe</td>
<td>52.908</td>
<td>3</td>
<td>163.167</td>
</tr>
</tbody>
</table>
*aAsymptotically F distributed.

While taking length as dependent variable, statistical difference was seen in every group. While taking head circumference as dependent variable, relationship between HC and Hb was marginally not statistically significant between no anaemia and mild anaemia group (p=0.011).

Table 8: Welch ANOVA test with length (cm) as dependent variable.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welch</td>
<td>33.585</td>
<td>3</td>
<td>55.145</td>
</tr>
<tr>
<td>Brown-Forsythe</td>
<td>34.516</td>
<td>3</td>
<td>117.978</td>
</tr>
</tbody>
</table>
*aAsymptotically F distributed.

Rest of combinations were statistically significant. While taking chest circumference as dependent variable, relationship between CC and Hb was not statistically significant between moderate anaemia and mild anaemia group (p=0.054). Rest of combinations were statistically significant.

Table 9: Welch ANOVA test with head circumference (cm) as dependent variable.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welch</td>
<td>39.166</td>
<td>3</td>
<td>59.554</td>
</tr>
<tr>
<td>Brown-Forsythe</td>
<td>37.269</td>
<td>3</td>
<td>232.814</td>
</tr>
</tbody>
</table>
*aAsymptotically F distributed.

Table 10: Welch ANOVA test with chest circumference (cm) as dependent variable.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welch</td>
<td>39.162</td>
<td>3</td>
<td>56.449</td>
</tr>
<tr>
<td>Brown-Forsythe</td>
<td>38.190</td>
<td>3</td>
<td>154.861</td>
</tr>
</tbody>
</table>
*aAsymptotically F distributed.

While taking MAC as dependent variable, relationship between MAC and Hb was not statistically significant between moderate anaemia and severe anaemia group (p=0.057) and also between no anaemia and mild anaemia group (p=0.025). Rest of combinations were statistically significant.

Table 11: Welch ANOVA test with mid-arm circumference (cm) as dependent variable.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welch</td>
<td>27.196</td>
<td>3</td>
<td>55.048</td>
</tr>
<tr>
<td>Brown-Forsythe</td>
<td>30.976</td>
<td>3</td>
<td>83.677</td>
</tr>
</tbody>
</table>
*aAsymptotically F distributed.

DISCUSSION

Anaemia

Present study showed that 53.75% women in their 3rd trimester were anemic, which closely mimic the WHO data but is about 11% more than the prevalence in Himachal Pradesh.36 In the present study mild, moderate and severe anaemic mothers were 22.25%, 28.25% and 3.25% respectively in comparison to Himachal data of 11.1%, 25.7% and 6.2% respectively.36 Present study shows more cases of mild anaemia and less of severe anaemia cases. Decrease in severe anaemia cases is a welcome sign but overall 53.75% cases of anaemia that too in 3rd trimester is worrisome. More input both from the people and health care system including policy makers is required to decrease this high prevalence of anaemia. Comparing measured and calculated Hb: the mean measured Hb value (obtained from automated blood cell analyzer method) was 10.68 gm/dl while the corresponding value derived from 3-fold conversion of Hct i.e. calculated Hb was 10.77 gm/dl. Pearson's correlation coefficient (r) was 0.938. But on doing limits of agreement method by Bland Altman, though the accuracy (bias) of the test method was acceptable but repeatability (precision) was not acceptable. So, both methods are not interchangeable in 3rd trimester of pregnancy. On comparing both methods of measuring Hb in different groups of no anaemia, mild anaemia, moderate anaemia and severe anaemia the results were the same as accuracy (bias) was acceptable but repeatability (precision) was not acceptable in all these cases. In severe anaemia group repeatability was the worst.

Various studies have been done on this matter in various countries and most of them they show that the assessment of anaemia using the 3-fold conversion between Hb and Hct is not accurate with very few show that this conversion is acceptable.19,27-29,31 Only one study has been done in India regarding validity of this conversion in pregnant females which shows poor correlation of this conversion factor.30 Therefore the standard 3-fold conversion between the two measures cannot be
considered as valid for the assessment of anaemia in the 3rd trimester pregnancy.

Multiple factors may be the cause of this like age and seasonal variations, physiological changes during pregnancy, errors of microhematocrit procedure such as errors in filling or sealing, reading errors and packing errors during the microhematocrit procedure etc. Also, there is a possibility of other unknown environmental factors and subject’s differences being the cause. So, due to the lack of agreement, the commonly assumed equivalent cut-off points for anaemia definitions which are even recommended by WHO need to be re-evaluated. This information is also of relevance for both clinical diagnosis and management of anaemia cases, as well as for descriptive and intervention studies on anaemia. Also, the imprecision in measurement of Hb by the Hct method may result in variations in calculation of mean corpuscular volume and MCHC which are important parameters in classification and thereby management of anaemia.

Effect of anaemia on newborn anthropometry: In this study while comparing anthropometric measurements between mild/ no anaemia group with severe anaemia group we found that all measurements for weight, length, chest circumference, head circumference and mid-arm circumference were less in severe anaemia group and this difference was statistically significant. Thus the present study showed that birth weight, height, head circumference, chest circumference and mid-arm circumference were affected by third trimester hemoglobin that too the most in severe anaemia cases which is consistent with previous studies.\(^{12,37-40}\) The association between maternal Hb and birth weight follows a U-shaped curve.\(^{41}\)

\[ Hb > 13.5 \, \text{gm/dl} \text{ or } > 3 \, \text{standard deviations higher than the mean of the reference population (i.e., a Hb concentration of } > 15.0 \, \text{gm/dl or a Hct of } > 45.0 \%, \text{is unusual and suggests inadequate plasma volume expansion (which can be associated with pregnancy problems including pre-eclampsia, poor fetal growth and preterm deliveries).}^{42,43} \]

In the present study we had only 12 cases (3%) with Hb > 13.5 gm/dl and none > 14.6gm/dl. Also, we didn’t include preterm delivery cases in our study. May be this is the reason we did not get less anthropometric parameters in no anaemia group.

Present study was conducted in a tertiary care hospital. Since many women in Himalach Pradesh deliver at home, the burden of anaemia can be expected to be much higher outside the hospital setting. By improving food intake, socioeconomic condition, literacy, qualitative antenatal care, early referral of risky cases, provision of iron and folic acid supplementation, parenteral iron therapy and blood transfusion whenever necessary we can reduce the incidence of anaemia in pregnancy and thereby reducing the ill effects on newborns.

Study limitation: this study was performed in a single center that too a tertiary referral center. Also, the sample size is not so big; it may not be representative of the whole population of the state or nation. Secondly, mothers were not categorized for some other maternal factors like low height and body mass index, which could contribute to neonatal anthropometric parameters. Also, we could not assess the hemoglobin levels in mothers during the first and second trimester and umbilical cord Hb. Not performing serum folate and Vitamin B12 levels, peripheral blood film microscopy and serum iron profile (for the confirmation and differentiation of anaemia) due to financial constraints was another shortcoming.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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