INTRODUCTION

Hemoglobin is a conjugated protein present in red blood cells. It transports respiratory gases from the lungs to the tissues and from the tissues to the lungs. The very high concentration of Hemoglobin within red cells increases its oxygen carrying capacity. The hemoglobin concentration is affected by factors such as age, gender and diet. Hemoglobin value at birth is normally 16- 22 g/dL. The adult males have hemoglobin value of 14-18 g/dL whereas for adult female is 12-16 g/dL for. The diet must contain adequate amount of iron for the RBCs to make hemoglobin. Iron deficiency anemia is known to be one of the important public health problems. Hence estimation of hemoglobin is gold standard in detection of this health problem [1].

There are many methods used to estimate hemoglobin level. One of them is Sahli’s Acid Hematin method. It is the most popular colorimetric method. However, this method is found to be unsatisfactory due to the difficulty in obtaining an accurate standard and the interference in the results by non-hemoglobin substances [2]. The other method used for estimation of hemoglobin is the hemocue method, in which, HemoCue Hemoglobin 201+ device and microcuvettes are used and where results are read within one minute. It is 99% accurate when compared to ICSH (International Council for Standardization in Haematology) method [3].

As part of Physiology practical curriculum at Melaka Manipal Medical College, we are using Sahlis method for estimation of hemoglobin for first year medical students. We conducted the present study in an attempt to compare the efficiency, accuracy and convenience of HemoCue method with that of Sahli’s Acid Hematin Method in estimation of hemoglobin. We also wanted to compare the haemoglobin level in a group of population with respect to their gender & BMI.
MATERIALS AND METHODS

The study was designed as a cross sectional study. A sample size comprising of 50 volunteers from Year 1 and Year 2 MBBS students of Melaka Manipal Medical College, Manipal were considered for the study. Informed consents were given to students who volunteered to be subjects of the study. A few drops of students’ blood was obtained for HemoCue’s Method and for Sahli’s Acid Hematin method. Results obtained from both methods were recorded and analysed for data interpretation.

HemoCue’s Method: HemoCue Hemoglobin 201+ DM was switched on and the pointed tip of the microcuvette was touched to the drop of blood. Microcuvette was allowed to be filled by capillary action. The filled microcuvette was placed in the black cuvette holder and analyzed. The patient test results displayed in g/dL were recorded.[3]

Sahli’s Acid Hematin Method: N/10 HCl was taken in a graduated tube up to 2 g%. When the second drop was collected, blood was drawn into the pipette exactly up to 20µl mark. (0.02 ml of blood). The blood was transferred in the pipette into the graduated tube containing HCl and mixed thoroughly. It is then left for 10 minutes to allow haemoglobin to be converted to acid hematin. Distilled water was added drop by drop, stirred thoroughly with the glass rod until the colour (brown) of acid hematin solution matched with the standards of comparator. Reading was noted when the colour of the solution exactly matches with the standard by holding the box against a natural source of light. Result was recorded in terms of g/100 ml when hematin solution attained the same colour intensity of standard [2].

The principle of measuring hemoglobin by Hemocue test is the conversion of hemoglobin to azidmethemoglobin which is measured by using wavelengths of 570nm and 880nm. While in Sahli’s acid hematin method the conversion of hemoglobin to acid hmatin by the addition of HCl, which is then estimated by comparing the color of the resulting solution with the known standard.

The data was obtained after performing Sahli’s Acid Hematin Method and HemoCue’s Method were analyzed statistically. Haemoglobin concentrations in the Sahli’s and Hemocue method were compared using “Bland Altman technique”.

RESULTS

Among the 50 subjects who participated in the study 21 were males and 29 were females. The age of the students ranged from 19-23 years. Hemocue method shows haemoglobin level which ranged between 7.9 g/dl and 16.1 g/dl with mean value of 13.5 g/dl. Sahli’s Acid Hematin method shows haemoglobin level which ranged between 11.8 g/dl ad 21.0 g/dl with mean value of 16.8 g/dl.

The number of male and female students with different ranges of hemoglobin level comparing the two methods is shown in the figure 1. Our study showed that majority of the male and female students had normal range of hemoglobin levels by both the methods. In hemocue method 6 male students and 7 female students were found to have below normal range which was not detected by Sahlis method. However in Sahli’s method 4 male students and 8 female students were found to have hemoglobin above normal range which was not seen in Hemocue method. The number of students having hemoglobin in normal range were the highest by both the methods.

![Figure 1: Number of male & female students with different ranges of hemoglobin level.](image-url)
When we compared the hemoglobin level in group of population with respect to their BMI we found majority of students to be in the healthy weight BMI category had normal hemoglobin level. Below table shows the total number of students with different hemoglobin levels by the two methods with their respective weight categories.

The Bland–Altman plot (Fig. 2) shows that 78% of the haemoglobin concentrations using the Sahli’s and Hemocue method are within acceptable limit (< 4gm%). A difference of less than 4gm% haemoglobin concentrations by the two methods is taken as an acceptable difference. However, 22% of the haemoglobin concentrations were much above the anticipated difference.

**DISCUSSION**

Iron deficiency anemia is the most prevalent nutritional disorder which affects approximately 30%-55% of adolescents of all over the world [4]. In our study majority of the students are having normal Hemoglobin level. However, quite a number of samples (26%) are having anemia which were detected by Hemocue method but not by Sahli’s method whereas, 24% of the samples, were found to have high Hemoglobin level only by Sahli’s method. There are many factors affecting the Hemoglobin level such as biological variation, age and sex, and race. Hemoglobin level tends to be lower in the evening than in the morning by the amount of 10g/L. By second decade of life, Hemoglobin concentration of female is lower than male because of inhibitory effect of estrogen on erythropoiesis. Anemia among women causes many serious health problems. However Hemoglobin level of males during adolescence are strongly influenced by testosterone and thus by age of sexual maturation. African have Hemoglobin values 5-10g/L lower than Caucasians [5]. It is likely that genetic factor is involved. But since our project is with Asians students we did not include race as the factor affecting the result. As we can see in Table 1, out of 50 students, the highest number of students (35) was categorized under healthy weight and the lowest number of students (1) was grouped under obese. Five students were grouped under underweight and nine were placed in overweight. The healthy weight students (BMI: 18.5-24.9), out of 35 students, 47% of them have a normal Hemoglobin level. A study conducted indicated the negative association of BMI to hemoglobin concentration which was observed among girls who were overweight and obese in both groups [6]. A similar study conducted showed that obese people had higher hemoglobin values than lean people [7]. Our research, showed that Hemoglobin level has no effect in an individual BMI. We indicate that individual with high Hemoglobin can have low BMI and vice versa.

**Table 1:** Number of students with different hemoglobin levels with their respective weight categories.

<table>
<thead>
<tr>
<th>Weight Categories</th>
<th>Below Normal Hemoglobin Level (Hemocue method)</th>
<th>Normal Hemoglobin Level (Hemocue method)</th>
<th>Above Normal Hemoglobin Level (Hemocue method)</th>
<th>Below Normal Hemoglobin Level (Sahli's method)</th>
<th>Above Normal Hemoglobin Level (Sahli's method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 18.5</td>
<td>Underweight</td>
<td>5</td>
<td>-</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>Healthy Weight</td>
<td>12</td>
<td>24</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td>25-29.9</td>
<td>Overweight</td>
<td>1</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>30 or over</td>
<td>Obese</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Kapil et al compared Hemocue method with Sahli’s method and they found that Sahli’s method underestimates hemoglobin
by 1.06g/dl compared to Hemocue method [8]. However, in our study we found that Sahli’s overestimated the Hemoglobin level when compared to the hemocue method. This could be due to the use of old apparatus in Sahli’s method. Sahli’s method has been known for several limitations. However, we would like to conclude that estimation of Hemoglobin by Hemocue method is more efficient, accurate and convenient when compared to Sahli’s Acid Hematin method.

REFERENCES

3. http://www.hemocue.com/international/Products/Hemoglobin/Technical_Specification #20ns-1156. html#HemoCue_Hemoglobin_201_System