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Comparative Study of Hemoglobin and Hematocrit by Manual and Automated Coulter Methods

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Abstract

A comparison of Automated and manual methods to determine the hemoglobin concentration and Hematocrit was done using a specified sample size of randomly selected patients. All the samples were subjected to Hb and Hematocrit estimation by using both manual and Automated methods. A significant difference between manual and automated Hct mehods was found. There was a good correlation between Cyanmeth-Hb and Automated method. The Cyanmeth-Hb is a gold standard for Hb estimation and also carries a biotoxic hazard. However, there was a statistically significant difference between Sahli's and cyanmeth- Hb method, but there was a good correlation. The Hct estimation by manual and automated methods showed an excellent relationship and no significant difference was noted between two methods and that can be used interchangeably.

Keywords: Sahli's, Cyanmeth-Hb, Hematocrit, Coulter method.

INTRODUTION

Hemoglobin is an iron containing metalloprotein in RBCs [1]. It is responsible for transportation of oxygen, carbon dioxide and proton between lungs and tissues. Hemoglobin below normal range for the age and sex of the person leads to 'Anemia'. It is commonly caused mainly by nutritional deficiencies, but also by inflammations, parasitic infestations, inherited and acquired disorders respectively. Hb-conc provides information about status of anemia in general population. Hb molecule, when fully saturated with O_2 (i.e four O_2 molecules combines with one Hb molecule, is called Oxy-Hb) [2]. One gram of Hb carries 1.34ml of oxygen. A molecule of Hb consists of two pairs of polypeptide chains (Globin) and four prosthetic heme groups, each containing one atom of ferrous iron. Located near the surface of molecule, the heme reversibly combines with one molecule of O_2 and CO_2. Main function of Hb is to transport O_2 from the lungs, where O_2 tension is high in the pulmonary capillaries (95% to 98% saturation). In tissues, where O_2 tension may be, as low as 20mm Hg, the O_2 readily dissociates from Hb and in this instance, less than 30% of the O_2 would remain combined to Hb [3, 4]. Various common methods of Hb estimation are Sahli's, Cyanmeth-Hb, Oxy-Hb, alkaline hematin and automated Coulter method, respectively.

Hb estimation is the routine and frequently performed screening hematological test of laboratory services. WHO has defined the lower limit of normal for Hb conc at sea level to be 12.0 gm/dl in Women and 13.0 gm/dl in men [5]. Hb content of blood in solutions may also be estimated by several methods, by measurement of its colour, erythrocyte volume fraction, specific gravity or its iron content respectively. These methods are colour or light intensity matching techniques, that measure inert pigments in blood with different degree of efficiency [6]. The type of method is selected based on its feasibility, cost effectiveness, simplicity, reliability, and easy to use in laboratory and in the field. Type of method and site of blood sample used is found to make a significant difference within subject variability of Hb concentration [7-9].

Table 1: Hemoglobin reference ranges for different age and sex

Age/Gender	Grams/dL)	Grams//L)
Newborn	16-23	160-230
Children	11-13	100-140
Adult males	13-17.5	130-175
Adult females	12-16	120-160

Grams/Deciliter

Hematocrit (Hct) is a test that measures % age of blood, comprising of RBCs and often referred to as packed cell volume (PCV) or Erythrocyte volume fraction. It is considered as an integral part of a person's complete blood count (CBC), along with Hb conc, WBCs and Platelet counts. Methods of estimation of Hct include two Manual (microHct and macroHct) and automated methods [9]. Cell counters also measure Hct by hematocrit (%) = (RBC x MCV)/10. In macroHct method, percentage of PCV is measured manually by Wintrobe's method. In this study, we also compared Hct estimation by manual macroHct and automated methods. 100 ml of blood with a Hct of 45%, the erythrocytes occupy only 45 ml. It is considered as an integral part of a person's CBC, along with Hb conc, WBCs and platelet counts respectively.

Table 2: Hct reference ranges for various age and sex groups

		Percents (%)	SI Units (L/L)	Percents (%)	SI Units (L/L)
Adults	Males	47	0.47	42-52	0.42-0.52
	Females	42	0.42	36-48	0.36-0.48
Children	Newborn	56	0.56	51-61	0.51-0.61
	1 Year	35	0.35	32-38	0.32-0.38
	6 Years	38	0.38	34-42	0.34-0.42

Micro-Hct is a gold standard method for Hct determination, but it is associated with problems, leading to inaccurate and imprecise measurements [10]. Spun Hct value is 1 to 3% higher than the Hct from automated instrument due to plasma trapped in between erythrocytes. In normal situation, spun Hct, however, may give spuriously higher results (upto 6%) in disorders like, polycythemia, hypochromic anemias, sickle cell anemia and burn patients due to increase in trapped plasma, compared to normal condition. Of course, insufficient centrifugation can also introduce high Spun Hct [11].

Comparison of analytical method for the determination of PCV are very essential in clinical laboratory practices, as it improves quality of health care through accurate and reliable clinical decision making from diagnostic results of suitable alternatives. Comparison is necessary because each method is expected to serve as a quality control measure for the other. This study also estimates analytical performance between the manual macro-Hct and automated methods for PCV determination. PCV test is usualy done to diagnose or evaluate anemia and polycythemia. Conditions that can lead to low PCV include, generalised bleeding, renal bleeding and immune mediated hemolysis respectively.

AIMS AND OBJECTIVES

1. To compare the hemoglobin estimation by various methods (Sahli's acid hematin method, cyanmeth-Hb method and non-cyanide method of automated cell counter).
2. To compare hematocrit estimation by manual wintrobe's method and automated cell counter.

MATERIAL AND METHODS

This hospital based study was undertaken to determine the comparison of hemoglobin and hematocrit by various manual and automated methods in the hematology laboratory. This comparative study was conducted with an EDTA mixed sample size of hundred on randomly selected patients over a period of one year. Hb levels in Gms were estimated by three methods, like Sahli's, Drabkin's and Automated coulter methods consecutively. Hematocrit was also estimated on the same samples using manual wintrobe's tube method and automated coulter method.

Statistical analysis: The study data was analyzed by using statistical software.

OBSERVATIONS AND RESULTS

The study involved randomly selected patients for comparison of Hb and Hematocrit by various manual and automated methods.

Table 3: Age wise distribution of Patients

Age Group(yrs)	No. of Patients	%Age
0-10	06	06%
11-20	06	06%
21-30	28	28%
31-40	17	17%
41-50	16	16%
51-60	15	15%
61-70	12	12%
Total	**100**	**100%**

Out of 100 cases, (06%) were between 0-10 years of age, (06%) of 11-20 years, (28%) 21-30 years, (17%) of 31-40 years, (16%) 41-50, (15%) of 51-60 years and (12%) belonged to 61-70 years of age group.

As per sex distribution, females were maximally involved, comprising a total of 55 patients (55%), while among the opposite sex was 45% respectively. (M:F ratio 9:11).

Table 4: Comparison of Mean Hb by different methods

Different methods of Hb	Mean
Mean Hb by Sahli's method	13.0 gm/dl
Mean Hb by cyanmethHb method	12.2 gm/dl
Mean Hb by automated cell counter	12.5 gm/dl

Table 5: Comparison of mean hematocrit by manual and automated methods

Mean hematocrit by automated cell counter	Mean hematocrit by wintrobe's method
36.88 %	37.21 %

General distribution of patients according to severity of anemia indicated that 56% of patients were having normal level of their hemoglobin, whereas mild degree of anemia was seen in 28% of patients, followed by moderate degree in 14% and anemia of severe degree in 2% of patients, respectively.

Table 6: Distribution of patients according to severity of anemia (Age gp 0-14 yrs)

Hemoglobin	No. of patients	Percentage
Normal Hb(11-13gm/dl)	05	62.5%
Mild anemia(10-10.9gm/dl)	02	25%
Mod anemia 7-9.9 gm/dl	01	12.5%
Severe anemia (<7.0 gm/dl)	0	0%
Total	**08**	**100%**

Out of eight cases (0-14 yrs), the normal Hb level was seen in 62.5% of cases, whereas anemia of mild degree, moderate degree and severe degree were seen as 25%, 12.5% and 0% respectively. Distribution of adult male and female patients according to severity of anemia (>15 yr's age) indicated that, out of 40 cases of adult male, normal Hb level was seen in 28 patients (70%), whereas, anemia of mild degree, moderate degree and severe degree were observed in 17%, 10% and 3% of patients, respectively. On the other hand, 50% of adult female patients had normal Hb, while remaining 50% showed anemia of mild degree as, 33%, moderate degree as15% and 2% as severe degree, respectively.

Table 7: Comparison of Mean Hb (18-42 yrs) Reproductive age group(females)

Age group in yrs	No. of patients	Mean Hb by Sahli's method	Mean Hb by cyanmeth Hb method	Mean Hb by automated cell counter
18-42	25	11.8 gm/dl	11.1 gm/dl	11.3 gm/dl

Comparison of mean Hb by different methods involved, 11.8gm% by (Sahli's Method), 11.1gm% (Cyanmeth-Hb Method) and11.3gm% by automated method) respectively.

Table 8: Mean ± SD of Hb results by Sahli's and Drabkin's method

Parameters	Sahli's method	Drabkin's method	P-Value	R(correlation coefficient)
Hb	13.0 ± 2.54	12.2 ± 2.54	0.0271	0.99

Table 9: Mean ± SD of Hb results by automated and manual methods (Drabkin's)

Parameters	Manual	Automated	P-value	R (correlation coefficient)
Hb	12.2 ± 2.54	12.5 ± 2.55	0.4056	0.99

The correlation coefficient for relationship between the Sahli's and Drabkin's method is calculated by using correlation coefficient formula, (r^2= 0.99). The mean ± SD of Hb result by Drabkin's method is 12.2 ± 2.54, whereas that of Sahli's method is 13.0 ± 2.54.

The correlation coefficient for relationship between the manual and automated method is calculated by using correlation coefficient formula (r^2= 0.99). Mean ± SD of Hb result by manual method is 12.2 ± 2.54, whereas that of automated method is 12.5 ± 2.55.

Table 10: Mean ± SD of hematocrit result by manual and automated methods

Parameters	Manual	Automated	P-value	R (correlation coefficient)
HCT	37.2 ± 7.2	36.8 ± 7.0	0.685	0.99

Correlation coefficient for relationship between the manual and automated method is calculated by using correlation coefficient formula (r^2= 0.99). The mean ± SD of Hct result by manual method is 37.21 ±7.2, whereas that of the automated method is 36.88± 7.09.

DISCUSSION

The study was aimed to compare the Hb-estimation and Hct by various manual and automated methods, in order to establish the efficacy and reliability of different methods. Lewis study showed that some of the methods of Hb estimation may have an error of + / - 20% or more, which when compounded with poor technique and thus making the method highly unreliable [12].

HiCN method is a gold standard method, but gradually, it has been phased out as a routine method, because of some factors like, handling, transportation and control of reagents like Cyanides. In our country, approximately 70% of the laboratories still use the manual HiCN method for Hb estimation in the rural areas [13].

Sahli's method in principle involves conversion of Hb to acid hematin and comparing visually, the color developed with that of standard tinted glass. Hb value is directly read from the graduated hemoglobinometer tube. This method has many in built disadvantages, like subjective visual color comparison and need for accurate pipetting of 20 microliter of blood. Estimation of only acid hematin formed, fading of comparator on prolonged use and poor sensitivity and reliability, respectively are matters of concern by manual Sahli's methods.

In Drabkin's method, Hb is oxidized to meth-Hb by potassium ferricyanide, that reacts with cyanide ions of potassium cyanide to form cyanmethemoglobin [14]. The Hb is estimated with the help of cyanmethemoglobin curve. Advantages of this method are as follows.

a) Error due to subjective visual matching is avoided as spectrophotometer is used and hence reading is precise and reliable.
b) Measures all forms of haemoglobin except Sulfhemoglobin.
c) Single step procedure using single reagent.
d) Cyanmeth-Hb formed, produces broad absorbent band at 530 nm.
e) Good stable Hb standards are available.

On comparison, Hb estimation by Sahli's and Cyanmet-Hb method showed a significant difference and this may be due to above mentioned disadvantages of Sahli's method of Hb estimation, corroborating with study by Salim M [14] *et al.,* Inter observational variations in Sahli's method was significant, but on comparing Sahli's and Drabkin's methods of Hb estimation, there was no significant difference observed in the study. Similarly, Patil P J *et al.,*[15] in their study, found that Sahli's method had lower values of Hb in capillary and venous blood as compared to HiCN method. Hb conc was lower in capillary blood than venous blood by both methods. For accuracy of Sahli's method, the correction factor should be considered to ensure comparability of results with the reference method. Among study patients, Hb estimation by the Sahli's and Drabkin's method showed a significant difference, but also a good correlation.

Table 11: Mean ± SD of Hb results by sahli's and Drabkin's method

Parameters	Sahli's method	Drabkin's method	Significance
Hb	13.0 ± 2.54	12.2 ± 2.54	P=0.0271 (<0.05)

On comparing, Hb estimation by non-cyanide method (Automated) and Drabkin's method did not show a significant difference. However, Correlation of these two methods was excellent (r^2 =0.40). The evaluation has shown it, to be as reliable and reproducible as HiCN for measuring hemoglobin at all concentrations. The reagents used in non-cyanide methods are non-bio hazardous and do not affect the reliability of data determination. Similarly, in study by Shah VB *et al.,*[16] non-cyanide methods of Hb estimation offer reliability of safe and quality Hb estimation and should prove useful for routine laboratory

use. Non cyanide methods are easily incorporated in hemoglobinometers by using very minute quantities of reagents and test sample. So, Hb estimation by Automated and Drabkin's method did not show a significant difference in our study.

Table 12: Mean ± SD of Hemoglobin results by Automated and Drabkin's method

Parameters	Automated method	Drabkin's method	Significance
Hb	12.5 ± 2.55	12.2 ± 2.54	P=0.4056

As mentioned earlier, hematocrit as a measurement of PCV is useful in any hematologic workup and also an important tool in quality control programs in the hematology laboratory. Incorrectly reported Hct result may bias clinical decision in follow up of patients, blood transfusion decisions, and in the diagnosis of severe anemia. Despite its significance, it has received far less consideration in research from the standpoint of its reliability than, have the measurements of Hb or RBC counts. So, micro-hct method is a gold standard method for hematocrit determination [15].

A comparison of automated and manual method, to determine the hematocrit for 100 patients is shown by mean ± SD (Table-12). The correlation coefficient for relationship between the manual and automated method is calculated by using Pearson's correlation coefficient formula (r^2= 0.99). The mean ± SD of Hct result by manual method is 37.21 ±7.2, whereas that of the automated method is 36.88± 7.09. These results are similar to the study by Audu I Stephen. The current study indicated that manual Hct is higher than automated Hct, however the difference is not statistically significant. It showed that Hct correlation between wintrobe's method and automated method was good. A study undertaken in southern Ethiopia on comparison between micro-Hct and automated method for determination of hematocrit by Gebretsadkan G [17] *et al.,* using Mindray BC-3000 plus (automated analyzer) revealed statistically significant difference (P<0.002), when mean difference between both methods (automation and manual) were compared. In our study, HcT estimation by the manual and automated method didn't show a significant difference.

Table 13: Mean ± SD of Hct results by manual and automated method

Studies	Manual	Automated	P-value
Present study	37.2 ± 7.2	36.8 ± 7.0	> 0.05
Audu SI [4]	34.5 ± 7.3	34.3 ± 6.8	>0.0.5

However, data obtained from this study indicated, a strongly positive correlation between two methods (r^2 =0.99). Audu SI *et al.,* also reported a comparable positive correlation coefficient (r^2=0.95), when both methods were compared. However, the gold standard method is micro-Hct method. Here, we used macro-Hct method to compare with automated method, because of nonavailability of micro-HcT method. In this study, both methods showed good correlationship. The imprecision in measurement of PCV by the manual method may result in variation in calculation of RBC indices, such as MCV and MCH, which are important parameters in the classification of anemia.

Mean Hb obtained by Sahli's, Cyanmeth-Hb and automated methods was 11.8 gm/dl, 11.1 gm/dl and 11.3gm/dl respectively in the age group of 18-42 yrs (reproductive age group of females).

There was significant difference between Sahli's and Cyanmeth-Hb methods (P value = 0.0271). No significant difference was found between Cyanmeth-Hb and automated methods (P value = 0.4056). So, this study indicated good correlation between Wintrobe's and Automated methods. However, no significant difference was found between Wintrobe's and automated methods (P value = 0.744).

CONCLUSION

The study showed, a good correlation between Cyanmeth-Hb and Automated cell counter method. However, Cyanmeth-Hb method is a gold standard method for Hb estimation. Hence, non-cyanide (automated) method can easily replace them, as it doesn't carry any Bio-toxic hazard. However, there was a statistically significant difference between Sahli's and Cyanmeth-Hb method, but, there was a good correlation. Sahli's method is easily available and highly useful in rural setups, remote areas and resource constraint settings. Hematocrit estimation by manual and automated methods showed an excellent correlation but, no significant difference and can also be used interchangeably. However, a correlation with the gold standard, Microhematocrit method is still warranted.

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