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Comparison of Hb and PCV Values in Manual Methods – A Prospective Study

R. Ananya[1] and M.P.Brundha*[2]

[1]Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, 600077 Tamilnadu, India.

[2]Associate Professor, Department of Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, 600077 Tamilnadu, India

ABSTRACT

Hemoglobin iron-containing metalloprotein expressed in red for all vertebrate blood cells (erythrocytes), as well as some invertebrate tissues. A healthy person has 12 to 20 grams of hemoglobin in every 100 milliliters of blood. Hematocrit is a blood test measuring the percentage concentration of red blood cells (RBC) in the blood. The measurement will depend on the number and size of cells in red bloods. It is part of the complete results of a person's blood count, along with hemoglobin concentration, white blood cell count, and platelet count. The volume of packed cells (PCV) is a measure of the proportion of blood formed by cells. The value is expressed as one percentage or fraction of blood cells. For example, a 40 per cent PCV means that in 100 milliliters of blood there are 40 milliliters of cells. This study is to compare the Hb and PCV values derived from direct manual and automated methods. Nowadays PCV values are estimated through automated methods. If there are any electrical supply problems, low budget labs, unavailability of the wintrobe's tube, in a practical college set up we have sahli's haemoglobinometer , with this we can be able to calculate the PCV valve. But our results show that the automated PCV values are higher than the manual calculated PCV values and the automated methods are more accurate to follow. Also we found that the manual calculated PCV method is not a better alternative for Automated PCV method.

KEY WORDS: HEMOGLOBIN, PACKED CELL VOLUME (PCV), MANUAL METHOD , AUTOMATED METHOD.

INTRODUCTION

Blood is a body fluid found in humans and nearly all the animals that carries nutrients and oxygen into the body's cells and carries the metabolic waste products away from the cells. Blood is made up of plasma and multiple cells in vertebrates-red blood cells , white blood cells , and platelets(Cooper, 2016). Hemoglobin is an element that produces oxygen in the red blood cells.Hemoglobin molecule consists of four subunits of globular protein of which each subunit consists of a protein chain that is closely associated with the non-protein heme component. The part of the heme consisting of the iron the oxygen binds to the oxygen binding capacity of hemoglobin is 1.34ml O2 per gram (Green, 2016). Therefore haemoglobin plays an important function in transport.

A healthy person consists of between 12 and 20 grams of haemoglobin in every 100 ml of blood(Ajibola, 2012). PCV also known as haematocrit is nothing more than the percentage amount of red blood cells in the blood, measured as part of a blood examination. This is usually 40.75 percent for men-50.3 percent, for women-44.3

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percent. It is considered as part of the complete blood count results of an ind(Callan et al., 1992)ividual, along with the concentration of haemoglobin, the count of white blood cells and platelet counts. The definition of hematocrit (hemato from the Greek haima = blood; critter from the Greek krinein = to separate) is the ratio of the volume of packed red blood cells to the total blood volume and is therefore often referred to as the volume of packed cells or PCV(Callan et al., 1992; Institute and National Cancer Institute, 2020).

A haematocrit examination of a blood can be a point of reference for its oxygen delivery capability(Greilich et al., 2000). Too high or too low levels of haematocrit may cause blood disease, dehydration or other medical conditions. Laboratory diagnosis of anaemia is based on the haemoglobin (Hb) concentration, the number of red blood cells and the haematocrit of packed cell volume (PCV) values(Hou et al., 2020). Anaemia is most easily and accurately calculated by calculating PCV percent using the haematocrit process, whereas the determination of Hb concentration provides precise information on the type of anaemia that has been found to be easy to calculate and can be done in most rural areas where Hb concentration determination methods are not available and rough estimates are made using observed PCV values, which is a much simpler and cheaper approach(Dahil, 2019). Hematocrit (PCV) values are widely used in rural African human medicine because they are simpler and cheaper to perform using manual techniques(Lokwani, 2013).

Estimating hemoglobin in the blood is widely recommended in different physiological and pathological conditions and as both diagnostic and prognostic test particularly in case of suspected anemia that can be caused by different factors(Nayak, Rai and Gupta, 2011). Hemoglobin measurement is now performed in several laboratories using Automatic Hematology Analyzers but also in many other laboratories using Sahli 's Method a.k.a(Organisation mondiale de la santé et al., 2003; Nayak, Rai and Gupta, 2011). Acid Hematin Method, Cyanmethemoglobin Method (CMG) a.k.a Drabkin 's Method widely used to evaluate the concentration of hemoglobin in the blood of patients(Chung et al., 2020). The theory of the Sahli method or acid hematin method is very simple that the hemoglobin present in RBCs is converted into acid hematin, which is a dark brown colored substance, when the blood is added to N/10 Hydrochloric acid (HCl)(Kawthalkar, 2012).The color of the shaped acid hematin complex corresponds to the concentration of hemoglobin in the blood and is matched to the norm which is a reference brown glass supplied in the Sahli 's apparatus by diluting with N/10 hydrochloric acid or distilled water until the acid hematin complex color matches the norm colour(Sood, 1985).

The role of technology in hemoglobin testing has led to the advancement of groundbreaking tools and hemoglobin concentration quantification techniques in patients(Whitehead et al., 2019) . For example, the use of an automated hemoglobin analyzer has allowed fast, accurate and reliable results in the field of hematology. An automated hematology or hemoglobin analyser is widely used to have high throughputs to analyze a range of red and white blood cells as well as blood sample levels of hematocrites and hemoglobins(Barbhuiya et al., 2020). Modern analyzers give a higher accuracy performance compared to manual methods, at a fraction of the cost. An automated analyzer's initial cost is high and routine maintenance, and the laboratory staff needed for the system will increase costs(Whitehead et al., 2019; Barbhuiya et al., 2020). In addition, suitable climate conditions are needed, making it an unacceptable choice for non-laboratory environments such as test sites for mobile blood donors and field-based anemia screening projects(P et al., 2012; Whitehead et al., 2019; Barbhuiya et al., 2020). The aim of the study is to compare the Hb and PCV values in manual methods.

MATERIAL AND METHODS

20 fresh blood samples were collected to estimate the Hemoglobin level and PCV value for performing both direct and automated methods. The Hemoglobin level was estimated in a manual method using Sahli's haemoglobinometer, where Hydrochloric acid converts hemoglobin to acid hematin, which is then diluted until the color of the solution matches that of the comparator block. The PCV value can be calculated in both manual and automated methods, but a direct manual method is also available. That is the Wintrobe's PCV method.The Wintrobe method is performed similarly except that the Wintrobe tube is smaller in diameter than the Westergren tube and only 100 mm long. EDTA anticoagulated blood without extra diluent is drawn into the tube and the rate of red blood cells decrease after 1 hour is measured in millimetres. PCV is calculated manually and in automation by multiplying 3 with Hemoglobin value
Equipments required for Sahli's method:

1. Peripheral blood collection
2. Sahli's haemoglobinometer comparator
3. Sahli's haemoglobinometer tube
4. Sahli's pipette or haemoglobin pipette
5. Glass dropper
6. Glass rod for stirring.

Figure 1: EDTA vacutanier blood collection tube

Sahli's method:

Peripheral blood collection: Peripheral Blood samples were collected in EDTA tube (Figure 1) from randomly 20

patients who were taking their routine blood examination at the Clinical Laboratory in Saveetha Dental Hospital in Chennai, after obtaining the consent from the respective individuals. Approval was given by the ethical committee for working on research involving humans in Saveetha Institute of Medical and Technical Sciences (SIMATS). The criterion for selecting the sample was the clinical request for Haemoglobin and Complete blood count. No restriction was made for age, sex, or clinical history of each patient under clinic care. All the procedures were carried out in the clinical laboratory of Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences.

Method to calculate Hb using sahli's acid hematin method by using sahli's hemoglobinometer :

1. Place N/10 HCl into the Hb tube upto 2 grams. The main principle of adding N/10 HCl is for the conversion of Hb to acid hematin which is brown in colour.
2. The peripheral blood sample collected has to be taken using sahli's pipette or haemoglobin pipette upto 20 microliter.
3. Now add the blood sample to the acid solution.
4. The prepared solution has to be stirred using a stirrer.
5. Allow to stand for 10 minutes.
6. Distilled water has to be added drop by drop till the solution matches to brown glass standard.
7. Take the reading of the lower meniscus from the graduated tube in grams.

The PCV value obtained manually by3 times of Hemoglobin with the standard calculation
(3X Hemoglobin value).(Gessese et al. 2020)

RESULTS AND DISCUSSION

As the results obtained from the automated method and calculated method (Table 1), PCV values were compared statistically by using Independent t test and the results of that shows a significant difference between the two methods. The automated value is higher than the calculated PCV value(Table 2). The difference was statistically significant.(P value <0.05)(Table 3). From our results, it shows that calculated PCV value cannot be used as an alternative for the automated method. As the recent advances showed, the automated values are more standardised than the manual methods by rectifying the manual and instrumental errors.

Hemoglobin is the protein molecule in red blood cells that brings oxygen from the lungs to the tissues of the body, and returns carbon dioxide to the lungs from the tissues(Callan et al., 1992). Hemoglobin is composed of four associated protein molecules (globulin chains) (Barbhuiya et al., 2020). Normal range of Hb for men is 13.5- 17.5 grams per deciliter and for women it is 12.0-15.5 grams per deciliter (Ajibola, 2012). Depending on your age and gender a normal hemoglobin level is 11 to 18 grams per deciliter (g / dL)(Chung et al., 2020). Yet a healthy range is from 7 to 8 g / dL. Your physician will only use enough blood to achieve this amount. Most patients with a blood transfusion level of between 7 and 10 g / dL do not need to(Callan et al., 1992).

Table 1. Shows Patients' hemoglobin value(Sahli's method), PCV values both automated and calculated methods

SAMPLES	HB VALUE (G%)	PCV VALUE (AUTOMATED)	PCV VALUE (CALCULATED)
1.	16	44	48
2.	14	44	42
3.	17	37	51
4.	8	31	24
5.	10.6	54	31.8
6.	12	39	36
7.	10.6	40	31.8
8.	11.8	54	35.4
9.	11.4	50	34.2
10.	9.6	43	28.8
11.	12	40	36
12.	5	25	21
13.	7.8	25	23.4
14.	12.4	45	37.2
15.	6.2	40	18.6
16.	4.2	22	12.6
17.	12.2	46	36.6
18.	8.2	39	24.6
19.	11	54	33
20.	8	40	24

Packed cell volume (PCV) is the calculation of the volume ratio occupied by the red cells to the volume of whole blood in a capillary, venous, or arterial blood sample(Kamat, 2011). After appropriate centrifugation, the ratio is measured 6,10 and expressed as a decimal fraction. PCV was directly determined by inserting the blood sample in the Automated Hematology Analyzer (KX-21N sysmex, USA)(Kamat, 2011; Geetha, 2017). The automated analyzer for sysmex hematology can be run on its own or connected to a filmmaking and staining unit for blood(Chandrasekar, 2012).

Sahli's haemoglobinometer is a tool used to assess the amount of hemoglobin in the blood(Alstead, 1940). In action the Swiss scientist H invented a hemoglobinometer in 1902. This is based on a calculation of the color of the blood being examined, which is treated with hydrochloric acid, with normal colour(Quinn, 1987). A hemoglobinometer is an instrument used by spectrophotometric analysis to assess the hemoglobin content in the blood. Portable hemoglobinometers provide simple and convenient measurement which is particularly useful in areas where there are no clinical laboratories(Quinn, 1987; Verma, 1999). The basic principle of sahli's haemoglobinometer is that the blood is mixed with N/10 HCl which results in the conversion

of Hb to brown-colored acid hematin. The solution is filtered until the comparator box matches its color with the brown colored glass. The Hb concentration is read straight away. This is an acid hematin process – a simple, obsolete procedure for semi-quantifying concentration of haemoglobin in the blood, in which dilute HCl causes a change in brown color compared to tinted glass levels(Bell and Mcnaught, 1944).

Table 2. Shows the sample distribution and mean and standard deviation of the PCV in both automated and calculated values. The mean PCV value in automated is higher than the PCV value calculated from the manual hemoglobin value.

Group Statistics

	PCV	N	Mean	Std. Deviation	Std. Error Mean
Percentage	PCV automated value	20	40.6000	9.31552	2.08301
	PCV calculated	20	31.5000	9.60691	2.14817

Table 3. Show the values of Independent t test. PCV value obtained through Automated analyser is higher than the calculated PCV value. The difference is also significant statistically. [t= 3.041 and P value 0.004 (<0.05)].

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Percentage	Equal variances assumed	.116	.735	3.041	38	.004	9.10000	2.99225	3.04250	15.15750
	Equal variances not assumed			3.041	37.964	.004	9.10000	2.99225	3.04231	15.15769

Suppose, low budget labs, problems in electrical supply, unavailability of the wintrobe's tube, in a practical college set up we have sahli's haemoglobinometer, with this we are able to calculate PCV value(Bell and Mcnaught, 1944). But sahli's haemoglobinometer also has various disadvantages like acid hematin can not be converted to sulfhemoglobin, methemoglobin, and carboxyhemoglobin. Additionally, fetal hemoglobin is not converted to acid hematin, and so this procedure is not appropriate for young babies(Hall, 1909).

CONCLUSION

From this study we conclude that the automated PCV values are higher than the manual calculated PCV values and the automated methods are more accurate to follow. Also we found that the manual calculated PCV method is not a better alternative for Atomated PCV method.

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Conflict of Interest: None to declare.

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