VOLUME-8, ISSUE-1, JANUARY-2019 • PRINT ISSN No 2277 - 8160

Original Research Paper

Pathology

COMPARATIVE STUDY OF HEMOGLOBIN ESTIMATION BY DIFFERENT METHODS

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ABSTRACT

Background:

Hemoglobin (Hb) estimation is one of the most frequently ordered investigations. Estimation of exact levels is important to stratify the grade of anemia and subsequently direct the necessary treatment. Conventionally, Hb has been estimated using colorimetric method, which is time tested and recommended by the WHO. Now-a-days, the automated method is also becoming a popular method in many hospitals. However, there are not many studies assessing the accuracy of the automated method over the gold standard.

Materials and methods:

We retrospectively analyzed hemoglobin values in 180 adult patient-samples (18 per batch in 10 batches). Hemolyzed samples were excluded from the study. Blood samples were drawn in vials having K3 EDTA anticoagulants. After a proper mixing, hemoglobin was estimated by automated Sysmex XS-800i. Parallel estimation for Hb content was done manually by spectrophotometer 4010.

Results:

Patients ranged from ages 20 to 40 years (M:F=102:78). The lowest value recorded by Sysmex XS-800i was 5.8 while the highest value recorded was 18.6 gm%. The mean hemoglobin concentration on Sysmex XS-800i was 12.89. The lowest, highest and mean values recorded by the cyanmethemoglobin method on photometer were 5, 18 and 13.49 gm% respectively. This showed a mean difference of 0.597 and with significant p-value of <0.001.

Conclusion:

The lowest values of Hb were similar in both the methods but the mean as well as the highest values differed significantly. Our study found an accuracy of 95.57% with the 5 part analyzer when compared to the gold standard colorimetric method.

KEYWORDS : Hemoglobin, Cyanmethemoglobin, Hemoglobin estimation, colorimetry, automated method, accuracy

INTRODUCTION-

Hemoglobin (Hb) is a porphyrin-iron protein compound that transports oxygen from the lungs to the body tissues where it is utilized for energy metabolism [1, 2, 3]. Hemoglobin estimation is of prime importance in medical investigations. The diagnosis of anemia is an important aspect in the practice of hematology [4]. The grading of anemia is based upon serum hemoglobin levels (Hb) as per the World Health Organization (WHO) definitions [5]. Haemoglobin has multiple functions: transport of oxygen from the lungs to the tissues, to facilitate oxidative phosphorylation in the mitochondria, carriage of carbon dioxide from the tissues to the lungs as carbaminohaemoglobin, buffering of hydrogen ions formed in the erythrocyte from the conversion of carbon dioxide into bicarbonate, nitric oxide metabolism[6]. Hemoglobin estimation, usually measured on venous blood or capillary blood and sometimes in clinical situations in arterial blood, is the most frequent laboratory investigation requested. [7]. For accuracy and reliability of the measurement, both sample collection and analysis technique are critical[8].Hemoglobin measured through different sources do show variability in values obtained. The reasons are several and include the instrument variability, type of blood samples and certain other factors [9]. Reference ranges for HGB concentration (according to WHO definition) are considered as 12–16mg/dL for women and 13–18mg/dL for men[10]. Different methods utilized for Hb estimation include acid haematin, photometric cyanmethemoglobin estimation and automated estimation with the help of counters[11]. The standard method for measuring hemoglobin (Hb) in human blood is the well-recognized HiCN method as recommended by the World Health Organization (WHO)[12]. It is based on photometric detection of cyanmethemoglobin, as an alternative to this technology, HemoCue has developed a photometric method based on the determination of azide met hemoglobin [13].The cyanmethemoglobin method works on the principle of conversion of hemoglobin to cyanmethemoglobin by the addition of potassium cyanide and ferricyanide whose absorbance is measured at 540 nm in a photoelectric calorimeter against a standard solution [14]. In India approximately 70% of laboratories still use direct cyanmethemoglobin method (HiCN) for hemoglobin estimation especially in rural areas [15].

MATERIALS AND METHODS-

We conducted this study with the aim to evaluate the accuracy as well as the suitability of photometric versus automated methods of Hb estimation.

180 adult patients sent for Hb estimation from outpatient clinics and wards of Era's Lucknow Medical College and Hospital, Lucknow were included in the study. Ten patients were selected on alternate days and a total of 18 batches were made (10 samples per batch). Hemolyzed samples were excluded from the study. Blood samples were drawn in vials having K3 EDTA anticoagulants. After a proper mixing, hemoglobin was estimated by automated Sysmex XS-800i. Parallel estimation for Hb content was done manually by spectrophotometer 4010. Twenty µL of well mixed blood sample was taken in 5 ml of Drabkin's reagent and incubated at 37 °C for 10 minutes. Absorbance was taken at 540 nm [16]. PARA-12 commercial control was also run with each batch. Automated method on Sysmex XS-800i is based on the electronic impedance principle for cell counting while lysing and colorimetry for Hb

estimation. In the manual method, Hb in the sample reacts with Drabkin's reagent to form cyanmethemoglobin, which is estimated photometrically at 540 nm with the help of a standard Hb solution[17].

RESULTS-

A total of 180 samples, ranging from age 20 years to 40 years (M:F=102:78), were analyzed. The lowest value recorded by Sysmex XS-800i was 5.8 while the highest value recorded was 18.6 gm%. The mean hemoglobin concentration on Sysmex XS-800i was 12.89. The lowest, highest and mean values recorded by the cyanmethemoglobin method on photometer on 4010 was 5, 18 and 13.49 gm% respectively This showed a mean difference of 0.597 and with significant p-value of <0.001. A significant difference was found in the mean values of colorimeter and 5 part (p<0.001) despite a significant correlation between these methods. Taking calorimeter as a gold standard, [18 ,19, 20, 21] accuracy of which is 100%, the calculated accuracy of 5 part analyser was 95.57%.

TABLE-1: A significant difference was found in the mean values of Hb obtained using colorimeter and 5 part (p<0.001)

Method	Mean	SD	Mean Diff.	t-value	p-value
Colorimeter	13.485	2.703	0.597	3.724	<0.001
5 part	12.888	2.266			

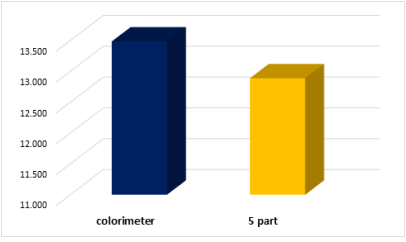

FIGURE-1: Histogram representation of mean Hb value in the colorimeter and the 5-part analyser.

DISCUSSION-

The present study, conducted at the Era's Lucknow Medical College, Lucknow, comprised a total of 180 routine samples of Hospital Lab Services, Department of Pathology. Out of the 180 samples, 102 were males and 78 were females. The age range was 25-45 years with a mean age of 35years.

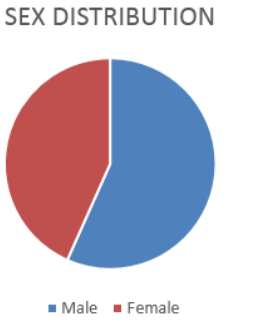

FIGURE-2: Pie chart representing the Sex distribution

Mayang et al, in their study concluded that haemoglobin concentration should be assessed with the direct cyanmethemoglobin method, the gold standard.[18] The photometer is easy to transport because it is small and light; it is battery operated and gives consistent results [22]. Photometric determination of haemoglobincyanide (HiCN) is recommended as the reference method.[23] If any other method is used in routine measurement (for example, photometric determination of oxyhaemoglobin or haemiglobinazide; iron determination), it should be adjusted to obtain a comparability with the haemiglobincyanide method.[24]

The spectrophotometric characteristics must conform to the specifications of the International Haemiglobincyanide Standard [25]. This method is recommended by the International Committee for Standardization in hematology. This is because of the uniform transformation of Hb to cyanmethemoglobin (except sulfhemoglobin) in this method and the availability of a firm and trustworthy standard [19].

The International Council for Standardization in Haematology (ICSH) in conjunction with Eurotrol, B.V. has released a new lot of the haemiglobincyanide or haemoglobin standard. [20]. Therefore in our study we have considered cyanmethhemoglobin method as a gold standard and calculated the accuracy of 5-part analyser accordingly.

The 5-part analyser's accuracy was 95.57%, which was less than the gold standard. Significant difference was found in the mean values of colorimeter and 5 part (p<0.001). That means the values of hemoglobin estimation for colorimeter and 5-part were not the same, despite the tests being done on the same sample. Though there was a significant correlation between the colorimeter and thw 5-part analyser, there is no such study in the literature exclusively for the comparision of hemoglobin estimation with 5-part analyser and a colorimeter.

In our study, we found that the correlation was better when the sample Hb was higher than lower levels of hemoglobin.

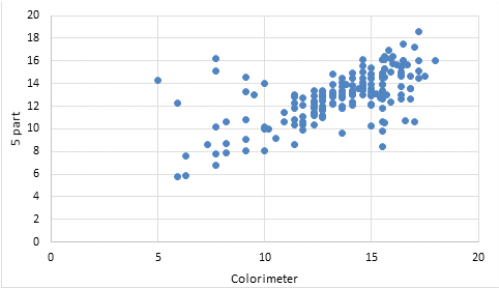

FIGURE-3: This figure shows that high level of hemoglobin correlates better than the low levels of hemoglobin in the present study.

In the Drabkin's method of haemoglobin estimation, haemoglobin is oxidised to methemoglobin by potassium ferricyanide, which reacts with cyanide ions of potassium cyanide to form cyanmethemoglobin. The haemoglobin is estimated with the help of cyanmethemoglobin curve.

The advantages of this method are (i) error due to subjective visual matching is avoided as spectrophotometer is used and hence reading is precise and reliable, (ii) measures all forms of haemoglobin except sulphaemoglobin. (iii) single step procedure using single reagent. (iv) cyanmethemoglobin formed produces broad absorbent band at 530 rim (v) good stable haemoglobin standards are available. [26]

CONCLUSION-

Our study showed that the automated method of Hb estimation is an alternative method to the standard colorimetric method. The lowest values of Hb was similar in both the methods but the mean as well as the highest values differed significantly. Our study found an accuracy of 95.57% with the 5 part analyzer when compared to the gold standard colorimetric method.

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