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Pathology

A Comparative Study on Haemoglobin Estimation of Drabkin's and Automated Analyzer Methods

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Abstract	Original Research Article

Haemoglobin, the red blood cell molecule that carries oxygen, is a vital element of circulatory system. Disorders of haemoglobin include Thalassemia syndromes, structural haemoglobin variants, anemia and polycythemia. Various laboratory methods are proposed for haemoglobin estimation. In our study, we compare automated analyzer and the standard Drabkin's method for haemoglobin estimation with 1000 samples that were collected randomly from the inpatient and outpatient departments. Results: The mean Hb concentration in Drabkin's method and automated analyzer in this study are 11.37 g/dl and 11.24 g/dl respectively. Automated method had given the hemoglobin values which are close to the gold standard Drabkin's method. Comparison analysis results of haemoglobin estimated by Drabkin's and Automated method showed non-significant difference in hemoglobin estimation. Correlation coefficient showed a maximum significance between Drabkin's and Automated method (r = 0.98). Conclusion: In this cross-sectional study of haemoglobin estimation we found that the automated analyzer method of haemoglobin estimation is a reliable, time saving and has a minimal manual workload with a better correlation of haemoglobin values with that Drabkin's method.
Keywords: Haemoglobin, Drabkin's method and automated analyzer method.

INTRODUCTION

Haemoglobin, an oxygen carrying pigment present in red blood cells of humans, is a protein that contains iron and porphyrin. Its protein part consists of 574 amino acids. It is formed by symmetric pairing of a dimer of polypeptide chains, the and β globins, into a tetrameric structural and functional unit. The chain is composed of 141 amino acids and the β chain has 146 amino acids [1]. In healthy adults, 95% of the Hb is Hb A ($\alpha 2\beta 2$) with small amounts (3.5%) of Hb A2 ($\alpha 2\delta 2$) and Hb F ($\alpha 2\gamma 2$) present. During embryonic development, "pre alpha" ζ globin chains contribute to embryonic Hb. During fetal development, β-like globin chains e and γ contribute to the Hb [2].

Structural haemoglobin (Hb) variants are based on a point mutation in a globin gene that produce a single amino acid substitution in a globin chain. Homozygous Hb C and Hb S (sickle cell disease) produce significant clinical manifestations, whereas Hb E and Hb D homozygotes may be mildly symptomatic. Although heterozygotes for these variants are typically asymptomatic, diagnosis may be important for genetic counseling. Thalassemia, in contrast, results from quantitative reductions in globin chain synthesis. Those with diminished β-globin chains are termed β-thalassemias, whereas those with decreased α chain production are called α thalassemias. Severity of clinical manifestations in these disorders relates to the amount of globin chain produced and the stability of residual chains present in excess. The thalassemia minor syndromes are characterized clinically by mild anemia with persistent microcytosis. Thalassemia intermedia (HbH disease) are presented with moderate anemia which may produce with clinical symptoms during a period of physiologic stress such as infection, pregnancy, or surgery. The thalassemia major syndromes produce severe, life-threatening anemia. Although haemoglobinopathies and thalassemias are two genetically distinct disease groups, the clinical manifestations of both include anemia of variable severity [3].

Anemia is of major health issue in many countries to be dealt with World Health Organization (WHO) defines the lower limit of normal for Hb concentration at sea level to be 12.0 g/dl in women and 13.0 g/dl in men [4]. The criteria for diagnosis of

anemia includes, a level of <12.0 g/dl in non-pregnant women and <13.0 g/dl in men [6]. It is a common hematologic disorder defined pathophysiologically as a decrease in the oxygen-carrying capacity of the blood resulting in tissue hypoxia and it often produces symptoms like fainting, fatigue, pallor, and difficulty in breathing [4]. Anemia is functionally defined as an insufficient RBC mass to adequately deliver oxygen to peripheral tissues [6]. Estimated prevalence of anemia in developing countries is 39% in children <5 years, 48% in children 5–14 years, 42% in women 15–59 years, 30% in men 15–59 years, and 45% in adults >60 years[7]. Anaemia reduces not only functional capacity and mobility of a person but also quality of life. However, many physicians continue to neglect the significance of anemias a serious clinical condition. Polycythemia, defined as central haemoglobin concentrations more than 20 g/dl and hematocrit levels more than 65% [8]. The usual range of haemoglobin in these individuals is between 18 and 20 gm/dl with haematocrit ranging from 49 to 55 percent [9]. Polycythemia is associated with hyperviscosity of blood, resulting in impairment of tissue oxygenation and perfusion and a tendency to form micro thrombi.

The measurement of Hb has traditionally relied on the services of a well-equipped clinical laboratory. Cyanmethaemoglobin method has been the gold standard for haemoglobin estimation [14]. A number of other methods are available such as haemoglobin color scale, Sahli's technique, Lovibond-Drabkin technique, Tallqvist technique, copper-sulfate method, HemoCue and automated hematology analyzers[15]. In spite of availability of many methods in Haemoglobin estimation, the search of reliable method still continues. The present work was undertaken to review Hb assessment methods that can work best from the developing country perspective.

MATERIALS AND METHODS
Type of study: It is a cross sectional study.

Duration of the study
November 2016 to March 2018, a period of 18 months

Nature of study
It is a cross sectional study on methods of hemoglobin estimation. Samples are received in the Hematology – central laboratory, Department of Pathology of Sri Venkateshwaraa Medical College, Hospital and Research Centre, Puducherry. Ethical clearance obtained from institutional ethical committee.

Sample size
Samples were collected from 1000 subjects whom were randomly selected. It includes both sexes with irrespective age groups, after obtaining written and informed consent. Data were collected from the request form.

Inclusion Criteria
The subjects included in the study are patients (IPD/OPD) of all clinical departments of Sri Venkateshwaraa Medical College Hospital & Research Centre.

Exclusion Criteria
• Clotted samples
• Patients who diagnosed as jaundice
• Leukemic patients.

Methodology
Method of sample collection
Blood samples was collected intravenously from the patients were drawn in the K2-EDTA (Ethylenediaminetetra acetic Acid) vacutainers, labeled & processed in the laboratory for the following test methods.

Cyanmeth – HB Method (Drabkin's method)
Procedure: 20 µl (0.02ml) of blood was added to 5 ml of Drabkin's solution in a test tube. It is mixed well and allowed to stand for 3-5 minutes. The reading of test & standard was taken in photoelectric colorimeter at 540 nm. The haemoglobin was estimated with the help of cyanmethaemoglobin curve.

Automated analyzer
Samples collected in the K2-EDTA vacutainers was analyzed in the automated analyzer (MINDRAY - 5parts) and estimated haemoglobin levels were noted.

Stastistical analysis
Statistical analysis was done in SPSS software version 19. For the baseline characteristics, continuous variables were summarized by mean and Standard deviation. Categorical variables were expressed by frequency (percentage %). Independent t' test was used to compare automated method with the gold standard Drabkin's method.

Pearson's correlation coefficient was used to compare automated method with the gold standard Drabkin's method. p value < 0.05 was considered statistically significant. p value < 0.001 was considered highly significant.

RESULTS
Table 1 showed that age distribution in the study population. Among 1000 patients, 0.7 % of our study group were under 1; 5.5% were between 1 and 14 years; 45.9% were between 15 and 44 years; 30 % were between 45 and 64 years; 17.9 % were above 65 years.

Table-1: Age distribution in the study population

Variable	Group	Percentage (%)
Age	Under 1	0.7
	1-14	5.5
	15-44	45.9
	45-64	30.0
	Above 65	17.9

In our study group, 51.5% were females and 48.5% were males as shown in the (Fig 1)
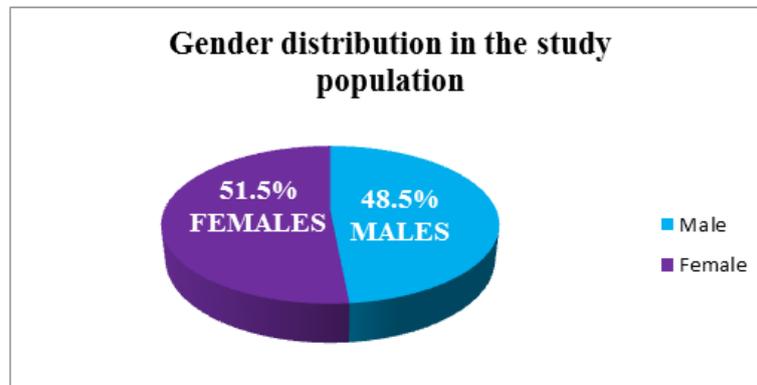

Fig-1: Gender distribution in the study population

On comparison of haemoglobin estimation by Automated analyzer and Drabkin's method showed almost similar value with a mean score of 11.2 and 11.3 which is non-significant (p= 0.263) as given in (Table 2).

Table-2: Comparison of haemoglobin estimation by automated analyzer and Drabkin's methods

Methods	Mean	Sd	Minimum	Maximum	Statistical Significance
Automated Method	11.246	2.493	2.5	18.6	T= 1.119 P=0.263 (Ns*)
Drabkin's Method	11.371	2.479	3	18.8	

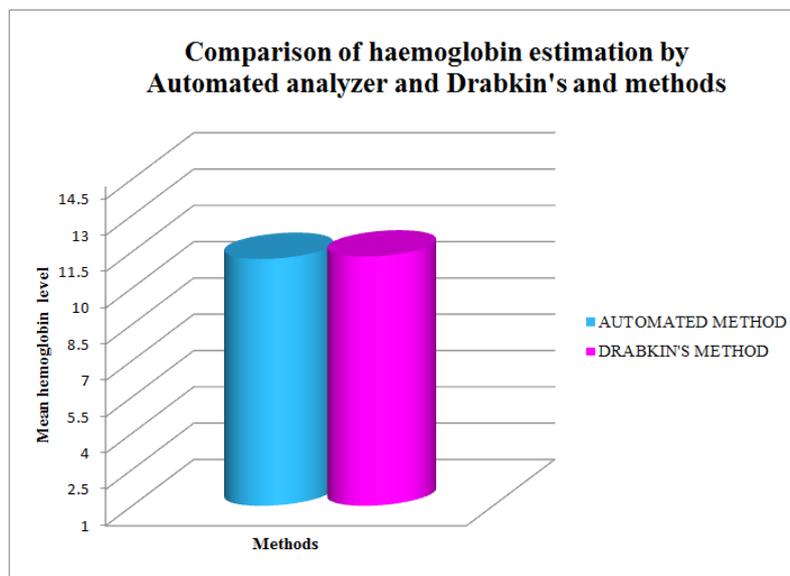*NS – Not significant, **HS- Highly significant

Fig-2: Bar graph showing Comparison of Automated analyzer, Drabkin's methods

In correlation analysis, Automated analyzer method showed a statistically higher correlation with r = 0.98 than Sahli's method with r = 0.97, when compared with Drabkin's method as shown in the Table 3.

Table-3: Correlation analysis with Drabkin's methods

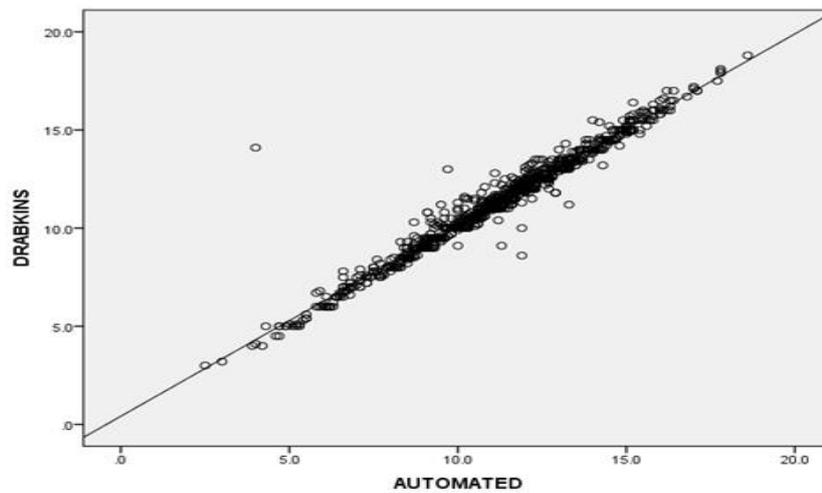| Methods	Correlation	Statistical Significance
Automated Method	R =0.98	P<0.001

Fig-3: Scatter plot showing correlation between Automated analyzer and standard Drabkin's method

DISCUSSION

Previous studies evaluating such laboratory instruments have provided little information on their reliability in specific situations. In the present study, 1000 samples were taken irrespective of age and sex to determine the haemoglobin level and to compare the methods used.

The mean Hb concentrations of Drabkin's method in our study were 11.37 g/dl in accordance with Mayang sari *et al*. [13] study as shown in the Table 4. The mean Hb concentration in Automated method is 11.24 g/dl in accordance with 11.86 g/dl in Samuel O Ike *et al*. [14], 13.7 g/dl in Ranjan *et al*.[15],10.2 g/dl Guy marino Hinnouho *et al*. [16] and 11.5 g/dl in Safia

Boghani *et al*. [17] as given in the Table 4. Using Drabkin's method as standard reference, we compared the automated method of hemoglobin estimation. The means of Drabkin's and Automated method showed non- significant difference in hemoglobin estimation which again proves, Automated values are mostly near equivalent to the standard Drabkin's method of hemoglobin estimation.

Correlation analysis showed a maximum significant r value (r= 0.98) for Drabkin's and Automated method which is in accordance with Chakravathy VK *et al*.[10] and Vinaya B Shah[18] *et al*. study in the (Table 4).

Table-4: Comparison of the results with previous studies

Study done by	Year	Sample size	Mean and standard deviation		Correlation Coefficient
			Automated Method	Drabkin's method	Automated & Drabkin's Method
Mayang Sari *et al.*	2001	121	-	13± 1.14	-
Samuel O Ike *et al*.	2010	60	11.86±0.3	-	-
Chakravarthy VK *et al*.	2012	2000	-	-	0.98
Ranjan et al	2016	750	13.7	-	-
Vinaya B Shah *et al*.	2016	200	-	-	0.96
Guy- Marino Hinnouho *et al*.	2017	1487	10.2 ± 1.3	-	-
Safia Boghani *et al.*	2017	213	11.5±0.9	-	-

A number of studies have compared haemoglobin estimated by different methods in order to establish the efficacy and reliability of the methods [12, 20]. Though automated hematology analyzers, require regular maintenance, control of calibration, trained personnel, they can provide high precision with less

than 1% error [19] and enable high-sample throughputs [21]. Time taken by manual method for a single sample requires longer time as compared to that of automated method which is approximately 3 minutes. The difference was pretty large, thus manual method

estimations consumes longer duration and causes fatigue of the technical staff [14].

Automated estimation is feasible and it can provide the whole blood picture and red cell indices. Now with increasing automation and increased requests for Hb estimation this method is highly suitable. This can provide less test timing, tests can be performed in batches and decrease the workload in the laboratory.

CONCLUSION

Haemoglobin estimation is one of the most important diagnostic tools done routinely in clinical practice. Our study concluded that, Haemoglobin measurements by automated method had a significant correlation with respect to the gold standard Drabkin's method. Automated method is suitable for testing a large group of samples at a time. When the Hb values given by automated analyzer is out of range, Drabkin's method can be used to standardize.

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