

# COMPARATIVE ANALYSIS OF AUTOMATED METHOD, SAHLI'S AND CYANMETHEMOGLOBIN METHOD FOR HEMOGLOBIN ESTIMATION USING ANOVA IN A TERTIARY CARE TEACHING HOSPITAL IN SRI GANGANAGAR, RAJASTHAN, INDIA

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## Abstract

**Background:** A quick screening technique for anaemia diagnosis that field workers in public health can employ is required. **Aim & Objectives:** To compare and contrast the automated cell counter, cyanmethemoglobin, and Sahli's Acid-Hematin methods of estimating hemoglobin for precision and accuracy. **Materials and Methods:** Patients visiting the out-patient and in-patient departments in the departments of medicine, surgery, obstetrics and gynecology, and pediatrics were chosen at random. Blood sample was taken after consent and Hemoglobin was estimated by all three methods. Results were analysed. **Result:** The results of the present study showed the accuracy of Sahli's method (69.20 %) to be less when compared to the accuracy of automated cell counter (94.5 %). The analysis of variance (ANOVA) revealed significant differences among the hemoglobin values estimated using the three methods. ( $f = 114.2960$ ,  $p$  value  $< 0.05$ )

**Conclusion:** In terms of accuracy, the cyanmethemoglobin approach outperforms both Sahli's acid-hematin and automated cell counting methods. Sahli's method when used to estimate the hemoglobin values at point of care must be accounted for deviation from the gold standard methods.

## INTRODUCTION

In affluent nations, it is more frequently observed as a complication of other disorders, such as hereditary reasons, blood loss from pregnancy or auto accidents, chronic disease-related anaemia, etc. The presence of pallor, tachycardia, weakness, dyspnea, and other symptoms is frequently noted during a clinical examination as the first indication of anaemia in any patient. But the majority of the time, laboratory tests are what alert us to an anaemic condition. Hemoglobin levels are one of the most accurate measures of anaemia and are frequently used to identify anaemic people and assess how well therapies are working. <sup>[1]</sup> The World Health Organization colour scale, Sahli's approach, HemoCue, and clinical examination for pallor are all frequently used techniques to measure hemoglobin in a community setting. Unfortunately, these

techniques have a number of drawbacks, from poor precision to complexity to expense. <sup>[2]</sup>

The present study was undertaken to compare and contrast the automated cell counter, cyanmethemoglobin, and Sahli's Acid-Hematin methods of estimating hemoglobin for precision and accuracy and to close the gap between the discovery of anaemia and the availability of medical care, a practical, affordable, and convenient method of hemoglobin.

## MATERIALS AND METHODS

At the out-patient and in-patient departments for the medical, surgical, obstetrics and gynaecology, and paediatric disciplines, respectively, patients were selected using chit method. Each patient underwent pre-evaluation, and a complete medical history was also obtained to rule out any confounding factors.

The randomization was assured to rule out selection bias.

Baseline characteristics that were compared included menstruation history, age, sex and socioeconomic status.

The automated cell counter's predicted hemoglobin values, cyanmethemoglobin, and Sahli's Acid-Hematin method were all compared using the chi-square test and ANOVA (Analysis of Variance). The results were given as mean  $\pm$  SD, and statistical significance was regarded as a p-value of 0.05 or less.

Graph Pad 2016 and SPSS Version 20.0 (IBM Statistics) were utilized to conduct the research.

## RESULTS

1000 patients of either sex and between 11-90 years of age were included in the study.

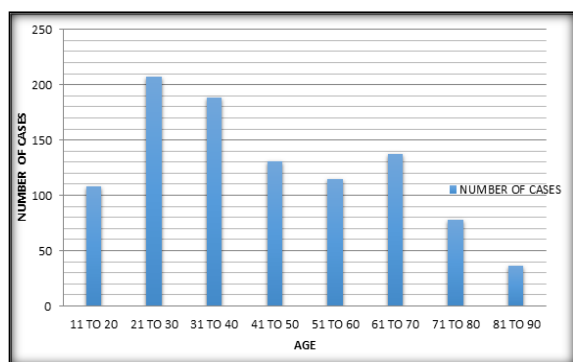


Figure 1: Age distribution of study participants

Mean age was observed as 43.83 years. Maximum number of cases were found to be in age group of 21-30 years whereas least number of cases were seen in the age group 81- 90 years. In this study, the youngest patient was 11 years old and oldest patient was 89 years of age.

In this study, majority of the cases were males i.e. 526 in number and 474 were females.

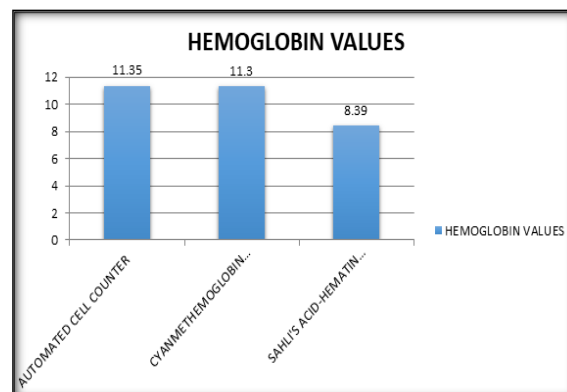


Figure 2 Graphical representation showing estimated Hemoglobin variation between automated cell counter, cyanmethemoglobin method and Sahli's acid-hematin method.

Closely related results were seen among automated cell counter ( $11.35 \pm 2.067$ ) and cyanmethemoglobin ( $11.30 \pm 2.03$ ) methods, while a huge difference was observed in estimation with Sahli's method ( $8.39 \pm 1.4759$ ).

Table 1: Shows Analysis of Variance among the Hemoglobin value estimated via automated cell counter, cyanmethemoglobin method and Sahli's acid-hematin methods

Analysis of Variance						
		Sum of Squares	df	Mean Square	F	p value
Automated Cell Counter	Between Groups	4268.669	999	4.273	114.2960	0.000
	Within Groups	.000	0	.		
	Total	4268.669	999			
Cyanmethemoglobin Method	Between Groups	4152.979	999	4.157		
	Within Groups	.000	0	.		
	Total	4152.979	999			
Sahli's Acid-Hematin Method	Between Groups	2176.133	999	2.178		
	Within Groups	.000	0	.		
	Total	2176.133	999			

The difference among the three clearly proved to be significant with ANOVA, f value: 114.296, p value <0.05.

Table 2: Accuracy of Automated cell counter with respect to Gold Standard Cyanmethemoglobin method.

		Cyanmethemoglobin method		
		Anaemia present	Anaemia absent	
Automated Cell Counter	Anaemia present	657	20	677
	Anaemia Absent	35	288	323
	Total	692	308	

Accuracy: 94.5 %

Table 3: Accuracy of Sahli's Method with respect to Gold Standard Cyanmethemoglobin method.

		Cyanmethemoglobin method		
		Anaemia present	Anaemia absent	
Automated Cell Counter	Anaemia present	691	297	988
	Anaemia Absent	11	1	12
	Total	702	298	

Accuracy= 69.20

## DISCUSSION

Sahli's acid hematin method can be done at the point of care, is quick, simple to use, affordable, and doesn't call for technical skill.

It does not measure all hemoglobins, such as oxyhemoglobin and sulfhemoglobin since they are not converted to acid hematin, and it is less accurate, lacks sensitivity and specificity and has high subjective variation in colour matching.

When calculating the hemoglobin values using the aforementioned three methods, it was found that the automated cell counter and cyanmethemoglobin methods produced results that were higher than those yielded by Sahli's acid-hematin method. When the automated cell counter and the acid-hematin approach developed by Sahli's were put vis-à-vis considerable differences were observed in the calculated hemoglobin values.

In terms of accuracy Sahli's acid-hematin method and automated cell counter both fell short of the cyanmethemoglobin approach. [Table 3 and 4]

In a similar study the accuracy of Sahli's method in diagnosis of anaemia in capillary & venous blood was 73.4% & 74% respectively.<sup>[3]</sup>

With a mean difference of 0.62 gm/dl (95% CI: 0.51 to 0.73, p value 0.01) in capillary blood and 1.1 gm/dl (95% CI: 0.92 to 1.26, p value 0.01) in venous blood, Sahli's approach had lesser values than the hemoglobincyanide method. According to a study conducted in 2013 Sahli's approach in calculating the capillary and venous blood hemoglobin had sensitivity and specificity of 83.7% and 90% respectively and can be used to detect anaemia (p value 0.01).<sup>[3]</sup>

In the current study, between the automated cell counting and the cyanmethemoglobin technique, there was only a small discrepancy in the calculated hemoglobin values. When cyanmethemoglobin was compared to the calculated hemoglobin values of the Sahli's acid-hematin method, there was a sizeable divergence. [Table 1]

When compared to HemoCue, Sahli's approach underestimated the values of hemoglobin in a study by Kapil et al. <sup>[4]</sup> There was a difference of 1.06 gm/dl. The values were also similar in Sahli's method also in the present study. While comparing Sahli's approach with the Coulter auto analyser in a study done by Natarajan S et al, <sup>[5]</sup> discovered results of reduced hemoglobin by 0.37 gm/dl utilising venous blood and 0.386 gm/dl in capillary blood.

In s study discovered p value of 0.0001 was discovered when compared Sahli's approach with cell counter, indicating a significant difference between the two methods.<sup>[6]</sup>

For accuracy of Sahli's method and automated cell counter method in the current study, the

cyanmethemoglobin method was considered as a gold standard for hemoglobin estimation as proved in a study done by H Radtke et al. <sup>[7]</sup>

The study conducted by Chandra D N et al showed results of comparing various hemoglobin estimation methods for accuracy were similar to the results of present study.<sup>[8]</sup> To maintain accuracy, both manual and automated methods should be standardised using controls on routine basis. The next best approach is to estimate hemoglobin using automated cell counts only.<sup>[9]</sup> The human and automated methods are significantly and favourably correlated.<sup>[10]</sup> Correct sample collection and procedure are crucial for accuracy.<sup>[11]</sup>

## CONCLUSION

This study clearly indicates that in contrast to Sahli's & automated cell counter methods, the cyanmethemoglobin method is the best in terms of accuracy and validity & could be used in hemoglobin estimation.

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