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Original Research Article

A STUDY ON EVALUATING THE RELIABILITY OF DIFFERENT HAEMOGLOBIN ESTIMATION METHODS: SAHLI'S, DRABKIN'S AND AUTOMATED ANALYZER

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ABSTRACT

Background: Anaemia is a major global health problem, especially in low- and middle-income countries. Accurate haemoglobin (Hb) estimation is crucial for diagnosis and management. Various methods exist, including Sahli's, Drabkin's (cyanmethemoglobin) and automated analysers, each with advantages and limitations. This study aimed to compare these three methods for Hb estimation performance.

Materials and Methods: This hospital-based, retrospective cross-sectional study was conducted on 1000 patients in a tertiary care hospital. Venous blood samples were analysed for Hb using three methods: Sahli's (acid hematin) method, Cyanmethemoglobin (Drabkin's) method, and an automated haematology analyser. Each method estimated Hb concentration photometrically, and results were recorded in g/dL for comparison.

Results: The majority were aged 15-44 years (45.9%), with females slightly predominating (51.5%) over males (48.5%). The Automated and Drabkin's methods showed comparable Hb levels (11.25 ± 2.49 g/dL vs. 11.37 ± 2.48 g/dL, $p = 0.263$), indicating no significant difference. In contrast, Sahli's method produced a significantly higher mean (11.89 ± 2.50 g/dL, $p < 0.001$) than Drabkin's method. Correlation analysis revealed a strong positive correlation between Drabkin's and automated methods ($r = 0.98$, $p < 0.001$) and a slightly lower but still significant correlation with Sahli's method ($r = 0.97$, $p < 0.001$).

Conclusion: Automated and Drabkin's methods showed strong correlation and reliability, while Sahli's method overestimated haemoglobin due to operator variability. Thus, Automated or Drabkin's methods are preferred for accurate, reproducible estimation.

Keywords: Anaemia, Colorimetry, Haemoglobins, Comparative study, Spectrophotometry.

INTRODUCTION

Anaemia continues to be a major public health issue worldwide. It affects roughly a third of people at some point in their lives and remains a common cause of illness and death, especially in low- and middle-income settings.[1] In routine practice, haemoglobin (Hb) level is the main laboratory measure used to identify anaemia, judge how severe it is, and plan treatment.[2] In clinical settings, accurate Hb estimation is thus essential for appropriate diagnosis, management, and epidemiological surveillance.

Several methods are available for estimating Hb concentration, each with its own advantages and limitations. The reference (or "gold standard") method widely recognised is the direct cyanmethemoglobin (HiCN) method (often associated with Drabkin's reagent) because it converts all Hb fractions to a single stable form and allows spectrophotometric measurement at 540 nm.[3] Its accuracy and reproducibility have been well documented.[4] However, in many resource-limited settings, the equipment cost, reagent hazards (potassium cyanide), and need for trained personnel

restrict its routine use. In contrast, the Sahli's acid-haematin method is a manual, inexpensive technique in which blood is converted to acid haematin and compared visually or photometrically with a comparator glass.[5] It has been widely used for screening of anaemia in peripheral and rural laboratories due to its low cost and minimal equipment.[6] A previous study reported that Sahli's method may underestimate Hb values compared with the cyanmethemoglobin reference method or automated analysers, possibly due to subjective colour matching, user variability, and a limited measurement range.[7]

Automated haematology analysers are increasingly common in modern laboratories. These instruments use either non-cyanide reagents or optical/impedance methods to estimate Hb as part of a full blood count profile.[8] Advantages include rapid throughput, minimal manual input, and good precision.[9] However, their higher cost, the need for regular maintenance, and reliance on uninterrupted electricity and reagent support can restrict their use in smaller or rural laboratories.

Different Hb estimation methods vary in cost, ease of use, and the chance of measurement error. Because of this, it is useful to see how manual techniques such as Sahli's and routine reference methods like Drabkin's compare with automated analysers in a real laboratory setting. This is especially important where limited resources require reliance on simple tools. Earlier studies have noted differences in mean Hb values, correlation, and accuracy of anaemia classification.[10,11]

Aim

This study aimed to compare the performance of Sahli's, Drabkin's, and automated analyser methods for Hb estimation.

MATERIALS AND METHODS

This hospital-based, retrospective cross-sectional study was conducted on 1000 patients at the Department of Pathology in a tertiary care hospital. The Institutional Ethics Committee approved this study, and written informed consent was obtained from each patient.

Inclusion Criteria

Patients of both sexes, irrespective of age (IPD/OPD), from all clinical departments were included.

Exclusion Criteria

Patients with clotted samples and those diagnosed with jaundice and leukaemia were excluded.

Methods

Sample collection

Venous blood samples were aseptically collected from all participants using K_2-EDTA vacutainers (ethylene diamine tetra acetic acid). Each sample was properly labelled and processed in the laboratory for Hb estimation using the following three methods: Sahli's method (acid hematin method), Cyanmethemoglobin method (Drabkin's method), and an Automated haematology analyser.

Sahli's method (Acid hematin method)

Hb estimation by Sahli's method was performed using a Sahli's hemometer. Approximately 20 µL (0.02 mL) of blood was added to N/10 hydrochloric acid, taken up to the mark "2" in Sahli's graduated Hb tube. The mixture was allowed to stand for at least 10 min for the conversion of Hb into acid haematin, resulting in brown colouration. Then, distilled water was added dropwise while stirring gently until the colour matched that of the comparator standard in natural light. The Hb concentration was read at the lower meniscus of the fluid column and recorded as g/dL.

Cyanmethemoglobin method (Drabkin's method)

Hb estimation by the cyanmethemoglobin method was performed using a photoelectric colourimeter. A volume of 20 µL (0.02 mL) of blood was mixed with 5 mL of Drabkin's reagent in a clean test tube. The mixture was allowed to stand for 3–5 min to ensure the complete conversion of Hb to cyanmethemoglobin. The absorbance of both the test and standard solutions was measured at 540 nm using a colourimeter. Hb concentration was calculated using a standard cyanmethemoglobin calibration curve, and the results were expressed in g/dL.

Automated haematology analyser method

Samples collected in K_2-EDTA vacutainers were also analysed using an automated five-part haematology analyser (MINDRAY). The analyser determined the Hb concentration photometrically as part of the complete blood count (CBC) analysis. The results were automatically processed and recorded as g/dL.

Statistical Analysis

Data are presented as mean ± standard deviation, frequency, and percentage. Hb obtained by Sahli's, Drabkin's, and the automated analyser was compared by a paired t-test. Pearson's correlation coefficient (r) was calculated to find the link with Drabkin's method. A p-value < 0.05 was considered significant. All tests were done using IBM SPSS v21.0.

RESULTS

Most participants were in 15–44 years (45.9%), followed by aged 45–64 years (30%). Females were a slightly larger share (51.5%) compared with males (48.5%). [Table 1]

Table 1: Age and gender distribution

Variable	Category	Frequency (%)
Age group (years)	< 1	7 (0.7%)
	1–14	55 (5.5%)
	15–44	459 (45.9%)
	45–64	300 (30.0%)
	> 65	179 (17.9%)
Gender	Male	485 (48.5%)
	Female	515 (51.5%)

Automated and Drabkin's methods showed almost similar Hb values (11.25 ± 2.49 g/dL vs. 11.37 ± 2.48 g/dL), with no significant difference (p = 0.263). Sahli's method, however, showed a higher mean Hb value (11.89 ± 2.50 g/dL) compared with Drabkin's, with a significant difference (p < 0.001). [Table 2]

Table 2: Comparison of Hb estimation by different methods

Comparison between methods	Hb (g/dL) (Mean±S.D)	p-value
Drabkin's method vs Automated method	11.25 ± 2.49 vs 11.37 ± 2.48	0.263
Drabkin's method vs Sahli's method	11.37 ± 2.48 vs 11.89 ± 2.50	<0.001

The automated analyser showed a slightly stronger correlation with Drabkin's method (r = 0.98, p < 0.001) than Sahli's method (r = 0.97, p < 0.001). [Table 3, Figures 1 and 2]

Table 3: Correlation analysis with Drabkin's method

Methods	Correlation	p value
Automated method	r =0.98	<0.001
Sahli's method	r =0.97	<0.001

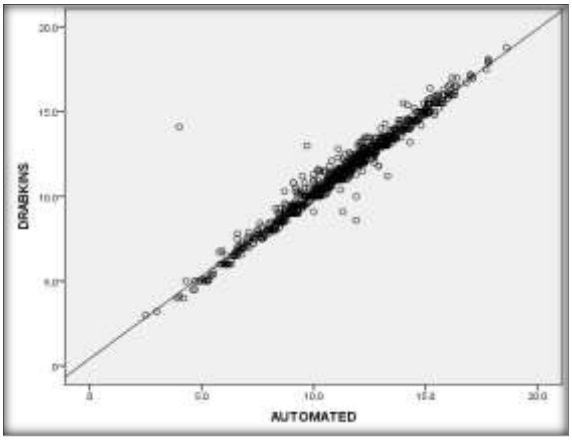

Figure 1: Scatter plot showing correlation between the automated analyser and the standard Drabkin's method

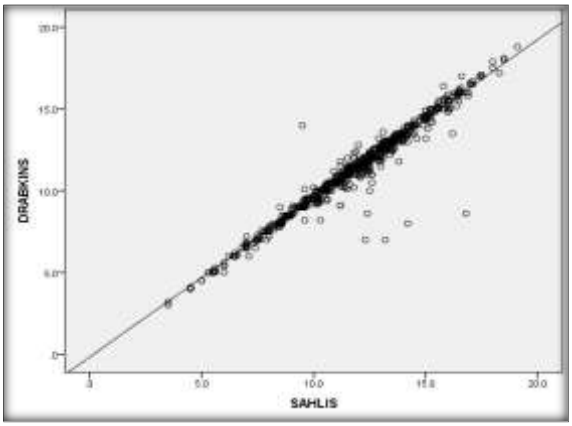

Figure 2: Scatter plot showing correlation between Sahli's method and the standard Drabkin's method

DISCUSSION

This study compared haemoglobin estimation by Sahli's, Drabkin's, and automated analyser methods to evaluate their accuracy and reliability in a tertiary care setting. The findings showed a strong agreement between the Automated and Drabkin's methods, while Sahli's method tended to overestimate haemoglobin values. Overall, the Automated method proved reliable for precise and large-scale haemoglobin estimation.

In our study, the mean Drabkin's value was 11.37 g/dL, which was lower than that in some reports but broadly comparable to that in many published studies. Sari et al. reported a mean Drabkin's Hb of approximately 13.0 ± 1.14 g/dL in a sample of 121 individuals.[12] Patil et al. reported a Drabkin's mean of 13.1 ± 1.0 g/dL (n = 173).[13] Several large studies have reported lower automated or Drabkin's values similar to our results. Agrawal et al. found means of 10.62 ± 1.9 g/dL (Drabkin's) and 10.64 ± 1.76 g/dL (Sahli's) in their study of 51 patients, while Hinnouho et al. documented an automated mean of 10.2 ± 1.3 g/dL in 1,487 samples.[14,15] Ike et al. reported an automated mean of 11.86 ± 0.3 g/dL (n = 60), and Boghani et al. observed an automated mean of 11.5 ± 0.9 g/dL (n = 213).[16,17] Ranjan et al. found a higher automated mean of 13.7 g/dL in 750 samples.[18] These studies collectively show that absolute Hb means vary by population characteristics, sampling frame, and laboratory factors.

In the present study, Sahli's method yielded a higher mean (11.89 g/dL) than Drabkin's method (by approximately 0.52 g/dL). Patil et al. reported Sahli's mean of 12.4 ± 1.12 g/dL compared with Drabkin's 13.1 ± 1.0 g/dL, showing variability across studies.[13]

Agrawal et al. found nearly identical means for Drabkin's and Sahli's (10.62 ± 1.9 vs. 10.64 ± 1.76 g/dL) with a correlation coefficient of 0.84, suggesting that while mean differences exist, the two methods may still correlate well in some settings.[14] Prashant et al. reported a lower correlation (r = 0.63) between Sahli's and Drabkin's in their cohort (n = 78).[19] This systematic positive bias with Sahli's is widely reported and is often attributed to its subjective colour-matching step and operator variability. In our larger sample (n = 1,000), the Sahli–Drabkin correlation was high (r = 0.97), indicating a consistent rank order despite the mean offset.

The automated method in our study had a mean Hb of 11.25 g/dL, which was close to several published automated values, such as those by Ike et al. (11.86 ± 0.3 g/dL) and Boghani et al. (11.5 ± 0.9 g/dL).[16,17] But the mean Hb value was lower than Ranjan et al. (13.7 g/dL) and higher than Hinnouho et al. (10.2 ± 1.3 g/dL).[18,15] Our study showed excellent correlation between automated and Drabkin's methods (r = 0.98). High concordance between automated analysers and cyanmethemoglobin reference values has been repeatedly reported. Chakravarthy et al. observed r = 0.98 in a large sample (n = 2,000), and Shah et al. reported r ≈ 0.96 in their 200-subject study.[20,21] Our findings support these previous observations, confirming that automated analysers serve as reliable alternatives to the reference cyanmethemoglobin method in routine practice.

The Sahli's method often yields higher mean haemoglobin values due to subjective visual colour comparison, making it prone to operator variability, lighting conditions, comparator ageing, and reagent inconsistencies, which can introduce systematic bias depending on local practice and training.[16] In contrast, Drabkin's cyanmethemoglobin and automated photometric methods are less operator-dependent and offer superior analytical reproducibility, explaining their stronger agreement observed across studies.[19,22]

Automated haematology analysers, despite the need for maintenance, calibration, and trained staff, offer high precision with error rates below 1% and support high-throughput work.[23] In contrast, Sahli's method takes 450 minutes for a batch, while automated analysis requires 3 minutes per sample, making batching essential to reduce technician fatigue.[16]

Limitations

The tests were carried out by different methods, which likely contributed to the higher Hb values obtained with Sahli's method. This limitation could be reduced by having three technicians test the same sample and using the mean Hb value, improving reproducibility.

CONCLUSION

The Automated and Drabkin's methods showed a strong correlation, supporting the reliability of Hb estimation. Sahli's method reliably gave higher values, due to operator-dependent variation. Although Sahli's method is useful in low-resource settings, Automated or Drabkin's methods are chosen when precision and reproducibility are required. Future work should focus on standardised operator training and evaluating cost-effective, non-cyanide alternatives for broader use.

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