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# Hemoglobin Determination by the Cyanomethaemoglobin, Sahli's Acid Hematin Method and Clinical Screening Criteria. A Comparative Study.

Paibool Lohsoonthorn \*

Busaba Matrakool \*\*

Charuay Srithong \*\*\*\*

Danai Danvivat \*\*\*

Ayut Dhamkrong-at \*\*\*\*\*

ไพบูลย์ โลห์สุนทร, บุษบา มาตระกูล, ดนัย ด้านวิวัฒน์, จรรยา ศรีทอง, आयुคัม ธรรมครอง आयคัม. การศึกษาเปรียบเทียบระดับฮีโมโกลบิน โดยวิธีไซแอนโนเม็ทฮีโมโกลบิน, ส่าห์ลีและการตรวจทางคลินิก จุฬาลงกรณ์เวชสาร 2524 กันยายน : 25 (5) : 995-1003

การศึกษาเปรียบเทียบค่าของฮีโมโกลบิน โดยวิธีไซแอนโนเม็ท ฮีโมโกลบิน วิธีส่าห์ลี และวิธีการตรวจทางคลินิกในประชากร 143 คน (ชาย 54, หญิง 58, เด็กชาย 13, เด็กหญิง 18,) ที่หมู่บ้านใน จังหวัดจันทบุรี ค่าฮีโมโกลบินโดยวิธีส่าห์ลีต่ำกว่าวิธีไซแอนโนเม็ท ฮีโมโกลบินเฉลี่ย 0.52 กรัมต่อ 100 ลูกบาศก์เซนติเมตร ค่าสัมประสิทธิ์แห่งสหสัมพันธ์สูง (0.84) ส่วนวิธีการประมาณค่าฮีโมโกลบินทางคลินิกได้ค่าสูงกว่าวิธีไซแอนโนเม็ท ฮีโมโกลบิน เฉลี่ย 1.4 กรัมต่อ 100 ลูกบาศก์เซนติเมตร ค่าสัมประสิทธิ์แห่งสหสัมพันธ์ต่ำ (0.49) สมการรีเกรสชันระหว่างค่า ฮีโมโกลบินโดยวิธีส่าห์ลี (X) และวิธีไซแอนโนเม็ท ฮีโมโกลบิน (Y) ได้แก่ "Y = 1.27 + 0.93 X" ค่าความไวและความจำเพาะของการทดสอบ โดยวิธีส่าห์ลี มีค่าเท่ากับ 71.4 เปอร์เซ็นต์ และ 85.2 เปอร์เซ็นต์ตามลำดับ ส่วนค่าการพยากรณ์ทางบวกค่า (54.1 เปอร์เซ็นต์) ค่าพยากรณ์ทางลบสูง (92.5 เปอร์เซ็นต์) การหาค่าฮีโมโกลบิน โดยวิธีส่าห์ลีค่อนข้างถูกต้อง บุคลากรสาธารณสุขสามารถนำไปใช้ในสนามได้ ส่วนวิธีการประมาณค่าทางคลินิกไม่ค่อยถูกต้องจะควรนำไปพัฒนาให้ดีขึ้นก่อนที่จะนำไปใช้ในสนาม

\* Department of Preventive and Social Medicine, Faculty of Med. Chulalongkorn U.

\*\* Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn U.

\*\*\* Prapok-Klao Hospital, Chanthaburi.

\*\*\*\* Department of Medicine, Faculty of Medicine, Chulalongkorn University.

\*\*\*\*\* Department of Pediatrics, Faculty of Medicine, Chulalongkorn University.

## Introduction

Anemia is one of the major problems affecting health and social well-being of the population of Thailand, intense measures have been taken to solve this problem.<sup>(2,4)</sup> The haemoglobin concentration is used as an index of severity, not only of the anemia itself but also of the underlying disease process. The cyanomethaemoglobin method is one of the photoelectric hemoglobinometers, being the method of choice in most hospital laboratories. Its advantages are time saving and accuracy.<sup>(5)</sup> The Sahli's acid hematin method is one of the visual hemoglobinometers. It is also a frequently employed procedure. Practically, anemia screening method is performed by clinical criteria for health workers in the field. There is obviously a need for a simple screening method in which public health workers can detect severely anemic individuals in the field.<sup>(8)</sup> The Sahli's acid hematin method and the clinical screening method are simple and may be used in the field, but their accuracy needed to be evaluated.

The aim of the present study was to determine the relationship of the hemoglobin values by the Sahli's acid hematin method and the cyanomethaemoglobin method, the sensitivity, specificity and predictive values of the Sahli's method and assessing the value of the clinical screening method by comparing with the cyanomethaemoglobin method.

The data were collected as a part of community health survey at Klung

district, Chantraburi province, in April 1981. The Klung district is situated on eastern region of Thailand, about 350 kilometres from Bangkok. The area is mainly agricultural fruitland.

## Materials and methods

All people residing in village number four, Klung district were eligible to participate in the study. The village has a total population of 336, one hundred and forty three of them (54 men, 58 women, 13 boys and 18 girls) participated in a half-day physical and laboratory examination. Duplicate blood samples were collected. Capillary blood was obtained from the finger pulp, the first drop or two were discarded. Whole blood hemoglobin values were determined by the Sahli's acid hematin method and the cyanomethaemoglobin method. The clinical screening examination was also obtained by a set of criteria in Figure 1. The total score of rating scales from the conjunctivae, nailbeds finger pulps, lips and buccal mucosae were added up. The scores below 8 were marked anemia, 8 to 11 were mild anemia and above 11 were normal. Each method of hemoglobin values was measured separately in a blind manner. The hemoglobins by the cyanomethaemoglobin method were used as the standard values. The regression equation and correlation coefficient were calculated.

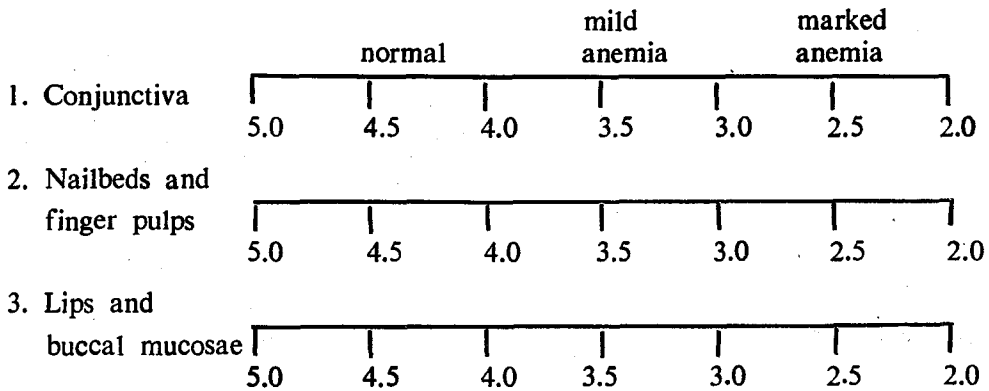
## Results

The hemoglobin values by the Sahli's method was lower than the cyanomethaemoglobin method with a

mean difference of 0.52 gm/100 ml, while the haemoglobin estimated by clinical examination was higher with a mean difference of 1.4 gm/100 ml (Table 1). The correlation coefficient between the Sahli's method and the cyanomethaemoglobin method was high (0.84) but it was low (0.49) between the clinical screening method and the cyanomethaemoglobin method. The regression line of hemoglobins between the cyanomethaemoglobin and the Sahli's method was shown in Figure 2. The regression equation was " $Y=1.27+0.93x$ ". The t statistic was 18.16 with 141 degrees of freedom,  $p < 0.001$ .

The sensitivity, specificity and predictive values of the Sahli's acid hematin method as compared to the standard (cyanomethaemoglobin) method were shown in Table 2. The sensitivity (71.4%) and specificity (85.2%) were relatively high. The predictive value positive (54.1%) was low, but the predictive value negative (92.5%) was high. The sensitivity, specificity and predictive values were varied with the cutting-off points for anemia (Table 3). The lower the cutting-off points were, the higher the sensitivity, specificity and predictive values were.

**Figure 1** Clinical screening examination of hemoglobin



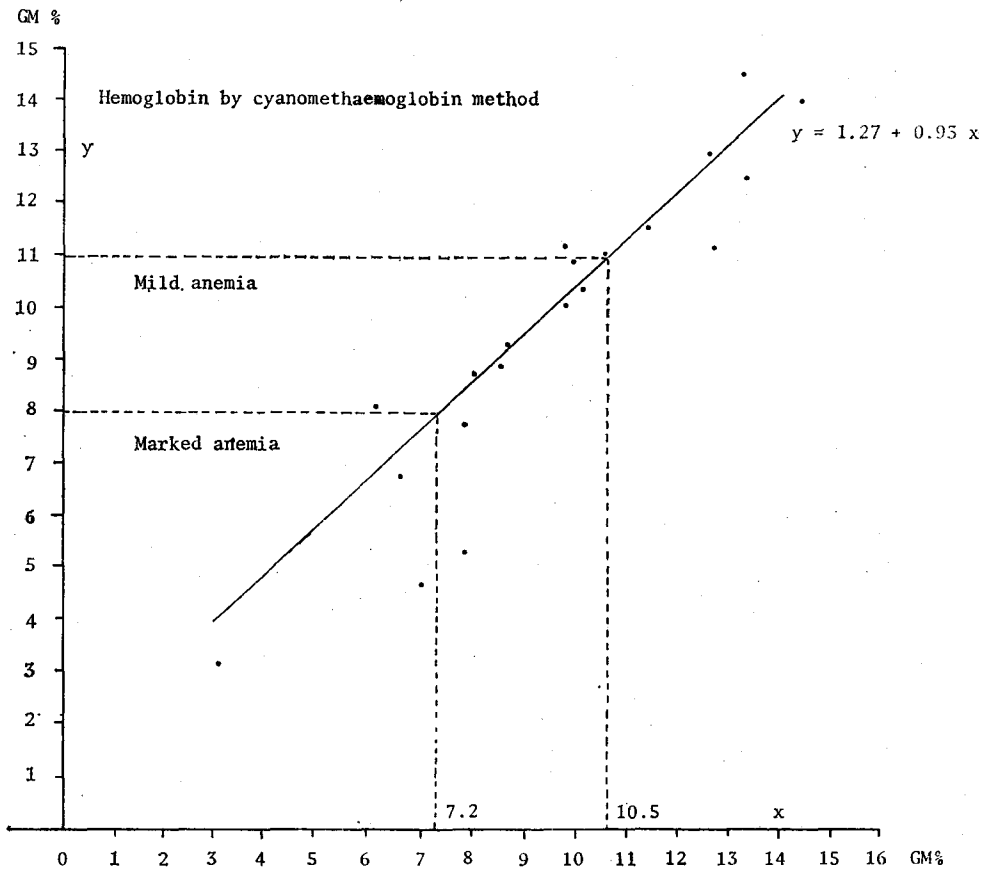
**Table 1** Basic statistical values in simple linear regression among cyano-methaemoglobin, Sahli's acid hematin and clinical screening methods.

Statistical values	Symbols	Computation of hemoglobin by Sahli's acid hematin method (x) and cyanomet-haemoglobin (y)	Computation of hemoglobin by clinical screening method (x) and cyanomet-haemoglobin (y)
1. Sample size	n	143	143
2. Summation of x values	$\sum x$	1,524.1	1,799
3. Summation of squares of x values	$\sum x^2$	16,671.07	22,748.0
4. Summation of y values	$\sum y$	1,598.1	1,598.1
5. Summation of squares of y values	$\sum y^2$	18,386.85	18,386.85
6. Summation of products of x values and y values	$\sum xy$	17,429.71	20,226.65
7. Regression coefficient	* $b_{y,x}$	0.9296	1.0523
8. y-intercept	**a	1.2674	2.0627
9. Mean of $x_i$	$\bar{x}$	10.6580	12.5804
10. Standard deviation of $x_i$	$S_x$	1.7344	0.9031
11. Mean of $y_i$	$\bar{y}$	11.1755	11.1755
12. Standard deviation of $y_i$	$S_y$	1.9269	1.9269
13. Correlation coefficient	r	0.8368	0.4932

$$* b_{y,x} = \frac{n \sum xy - \sum x \sum y}{n \sum x^2 - (\sum x)^2}$$

$$** a = \frac{\sum y \sum x^2 - \sum x \sum xy}{n \sum x^2 - (\sum x)^2}$$

Figure 2 Regression line of hemoglobins between cyanomethaemoglobin and Sahli's acid hematin method



Hemoglobin by Sahli's acid hematin method

$H_0 : \beta = 0, t = b/S_b$  with  $n-2$  df .....(1)

$b$  = regression coefficient = 0.9296

$S_b$  = standard error of the estimate for the slope = 0.0512

$t = 0.9296/0.0512 = 18.16$  with 141 d.f.,  $P < 0.001$

There is a relationship between the hemoglobins determined by cyanomethaemoglobin method and the hemoglobins determined by Sahli's acid hematin method.

**Table 2** The sensitivity, specificity, and predictive values of the Sahli's acid hematin method as compared to the standard (cyanomethaemoglobin) method, using below 10 gm% as cutting-off point for anemia.

		Cyanomethaemoglobin method		
		Positive	Negative	Total
Sahli's acid hematin method	Positive	20 True positive	17 False positive	37 All positive test
	Negative	8 False negative	98 True negative	106 All negative test
Total		28 All disease persons	115 All non-disease persons	143 Total

1. **Sensitivity** is the percentage of people with the disease who are detected by the test.

$$\text{Sensitivity} = \frac{\text{True positive} \times 100}{\text{All disease persons}} = \frac{20 \times 100}{28} = 71.4\%$$

2. **Specificity** is the percentage of people without the disease who were correctly labelled by the test as not diseased.

$$\text{Specificity} = \frac{\text{True negative} \times 100}{\text{All non-disease person}} = \frac{98 \times 100}{115} = 85.2\%$$

3. **Predictive value positive** is the likelihood that an individual with a positive test has the disease.

$$\text{PV} + \text{VE} = \frac{\text{True positive} \times 100}{\text{All positive tests}} = \frac{20 \times 100}{37} = 54.1\%$$

4. **Predictive value negative** is the likelihood that an individual with a negative test, does not have the disease.

$$\text{PV} - \text{VE} = \frac{\text{True negative} \times 100}{\text{All negative tests}} = \frac{98 \times 100}{106} = 92.5\%$$

**Table 3** The sensitivity, specificity and predictive values of the Sahli's acid hematin method at various anemic levels.

Screening levels for anemia.	Cyan+ve Sahli +ve	Cyan+ve Sahli -ve	Cyan-ve Sahli +ve	Cyan-ve Sahli -ve	Total	Sensitivity	Specificity	Predictive value+ve	Predictive value-ve
8 gm %	7	1	1	134	143	87.5 %	99.3 %	87.5 %	99.3 %
9 gm %	11	3	3	126	143	78.6 %	97.7 %	78.6 %	97.7 %
10 gm %	20	8	17	98	143	71.4 %	85.2 %	54.1 %	92.5 %



## Discussion

This study shows that there is a relationship between the Sahli's acid hematin method and cyanomethaemoglobin method. The hemoglobin value on the visual hemoglobinometer (Sahli's) differs from the hemoglobin value on the photoelectric hemoglobinometer. On the average, we are "under-reading" with Sahli's hemoglobinometer. We should make a correction on all of our future Sahli's readings by adding 0.5 gm% to our readings or calculate the hemoglobin values from the regression equation. The estimation of hemoglobin values by the clinical screening methods was inaccurate. Since the correlation coefficient between the clinical estimation and the cyanomethaemoglobin method was relatively low. The clinical screening method is not reliable. It should be improved by using photographic color scale. The cutting-off point for anemia may be set anywhere. If the screening level for anemia is set lower, there will be poorer sensitivity and better specificity.<sup>(3,6)</sup> The sensitivity, specificity and predictive values in Table 3 were all increasing when the screening levels for anemia were lower. These were due to the change of anemic levels of the standard test and the screening test simultaneously. Vecchio (1966)<sup>(7)</sup> pointed out that the sensitivity, specificity and predictive values were also influenced by the prevalence of disease.

## Conclusion

The study of the relationship of hemoglobin values among the cyanomethaemoglobin method, the Sahli's acid hematin method and the clinical screening method was carried out in a village in Eastern Thailand. The hemoglobin values by the Sahli's method were highly correlated with the cyanomethaemoglobin method. It was quite accurate and could be used by the public health workers in the field. The clinical screening method was inaccurate. It should be improved by photographic color scale before using in the field.

## Acknowledgements

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