

## **Methemoglobin and Sulfhemoglobin Levels in Students of Walailak University**

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### **ABSTRACT**

Methemoglobin (MHb) and Sulfhemoglobin (SHb) are abnormal oxygen-carrying protein hemoglobins (Hbs) in blood circulation which can be used to represent the degree of pollution in the surrounding environment including air, food and drinks. The MHb is an oxidized form of hemoglobin (Hb) in which the ferrous ion ( $\text{Fe}^{2+}$ ) in the heme group is oxidized to the ferric ion ( $\text{Fe}^{3+}$ ) by oxidizing agents mostly from the air, food, drinks and drugs. The SHb is a sulfurated form of Hb derived from hydrogen sulfide ( $\text{H}_2\text{S}$ ) and sulfur dioxide ( $\text{SO}_2$ ) gas contained in the air and drugs found in sulfur therapy, respectively. These types of Hb derivatives are unable to bind and carry oxygen to the tissues of the body. This makes inspiration insufficient and may cause cyanosis in increased amounts. The aim of this study is to measure MHb and SHb levels in Walailak University students. EDTA blood samples were collected from 200 students. The MHb and SHb were analyzed by spectrophotometry. The results revealed that the mean MHb and SHb levels of the 200 subjects were  $1.29 \pm 0.57$  % and  $0.66 \pm 0.28$  %, respectively. The level of MHb and SHb in population of Walailak University was very low compared to people in large cities such as Bangkok. The study proves that the environment of Walailak University remains clean, good and fresh.

**Keywords:** Methemoglobin (MHb), Sulfhemoglobin (SHb),  
Spectrophotometry

## INTRODUCTION

Methemoglobin (MHb) and Sulfhemoglobin (SHb) are 2 kinds of abnormal hemoglobins (Hbs) in blood circulation which have the properties to be used to represent the degree of pollution of the surrounding environment including air, food and drinks [1]. The MHb is an oxidized form of hemoglobin (Hb) in which the ferrous ion ( $\text{Fe}^{2+}$ ) in the heme group is oxidized to the ferric ion ( $\text{Fe}^{3+}$ ) by oxidizing agents mostly from the air, food, drinks [2] and drugs [3,4]. It is chocolate brown in color [5]. The NADH-dependent enzyme methemoglobin reductase (diaphorase I) is responsible for converting methemoglobin back to hemoglobin [6]. The SHb is a sulfurated form of Hb derived from hydrogen sulfide ( $\text{H}_2\text{S}$ ) [7] and sulfur dioxide ( $\text{SO}_2$ ) [8] gas contained in the air and drugs found in sulfur therapy, respectively [9-13]. Another possible cause is occupational exposure to sulfur compounds [14]. The condition generally resolves itself with erythrocyte (red blood cell) turnover, although blood transfusions can be necessary in extreme cases. The pigment is a greenish derivative of hemoglobin which cannot be converted back to normal, functional hemoglobin [15]. It causes cyanosis even at low blood levels [16]. Both Hb derivatives are unable to bind and carry oxygen to the tissues of the body. This makes inspiration insufficient and may cause central cyanosis in large amounts [17,18]. There are several methods that have been developed to measure the levels of MHb, SHb and other derivatives of Hb using spectrophotometry [19-25].

The purpose of this study is to evaluate the pollution of the surrounding environment of the accommodation at Walailak University, Nakhon Si Thammarat province by means of determination of MHb and SHb levels in the blood of students who are living and learning at the campus using spectrophotometry.

## MATERIALS AND METHODS

### Sample Collections

Blood samples were collected from 200 Walailak University students (100 males and 100 females). Three ml of blood was drawn by venipuncture from each student using EDTA as an anticoagulant. The MHb and SHb were analyzed by spectrophotometry for determination of the levels of individual Hb. All samples were analyzed within 1 h after blood collection.

### Determination of Methemoglobin (MHb) [26]

Blood (0.2 ml) was added to a mixture of phosphate buffer saline (PBS, 4 ml) and 5 % Triton X-100 solution (6 ml) and mixed thoroughly. The solution was divided into 2 parts, defined as solution "A" and "B".

Solution "A" was measured for MHb at 630 nm, defined as  $\text{OD}_1$ . One drop of KCN solution was then added, and the absorbance of solution "A" again measured at 630 nm, defined as  $\text{OD}_2$ , using PBS-5 % Triton X-100 as a blank.

Solution “B” was measured for total Hb by adding 1 drop of 1 %  $K_3Fe(CN)_6$  solution, mixed and allowed to stand at room temperature for 5 min. The mixture was then measured at 630 nm, defined as  $OD_3$ . One drop of 1 % KCN solution was added and the reaction mixture which was then measured again at 630 nm ( $OD_4$ ).

MHb was determined by the equations:

$$OD_3 - OD_4 = \text{Total Hb} = 100 \% \quad (1)$$

$$OD_1 - OD_2 = \text{MHb} \quad (2)$$

$$\% \text{MHb} = [(OD_1 - OD_2) / (OD_3 - OD_4)] \times 100 \% \quad (3)$$

**Determination of Sulfhemoglobin (SHb) [26]**

Blood (0.1 ml) was added to 10 ml of a 5 % Triton X-100 solution. The reaction was mixed and measured at 620 nm. The result was OD of total Hb which was defined as “ $A^{620} HbO_2$ ”. Then, 1 drop of 1 % KCN solution was added and the solution mixed. The mixture was incubated at room temperature for 5 min prior to measuring the absorbance again and defined as “ $A^{620} SHb$ ”. The reaction mixture was measured again at 578 nm and defined as “ $A^{578} SHb$ ”, against a blank (5 % Triton X-100 solution).

SHb was determined by the equations:

$$\% \text{SHb} = 2 \times (A^{620} \text{SHb} / A^{620} \text{HbO}_2) \times 100 \quad (4)$$

$$A^{620} \text{HbO}_2 = A^{578} \text{SHb} / \text{Conversion factor} \quad (5)$$

$$\text{Conversion factor} = A^{578} \text{SHb} / A^{620} \text{HbO}_2 \text{ of normal blood}^* \quad (6)$$

\*Normal blood was collected from people who live in a normal or clean environment.

**RESULTS AND DISCUSSIONS**

**Methemoglobin (MHb) Levels in 200 Students of Walailak University**

Determination of MHb by spectrophotometry is based on the fact that a molecule of MHb has a maximum absorption at 630 nm which will disappear when KCN is added to the solution. Addition of a solution of  $K_3Fe(CN)_6$  will convert oxyhemoglobin ( $HbO_2$ ) and deoxyhemoglobin (Hb) into MHb. The MHb levels from the 200 samples are shown in **Table 1**. There was no significant difference between males and females. The average (mean) percentage of Methemoglobin (% MHb) was  $1.29 \pm 0.57 \%$  with a range from the lowest of 0.00 % to the highest of 2.88 %.

**Table 1** Percentage of Methemoglobin (% MHb) in 200 Walailak University students.

Gender	N	MHb (%)		
		Mean $\pm$ SD	Min	Max
Male	100	1.26 $\pm$ 0.55	0.00	2.32
Female	100	1.32 $\pm$ 0.58	0.19	2.88
Total	200	1.29 $\pm$ 0.57	0.00	2.88

**Sulfhemoglobin (SHb) Levels in 200 Students of Walailak University**

Determination of SHb by spectrophotometry is based on the fact that the molecules HbO<sub>2</sub> and SHb have a maximum absorption at 620 nm. HbO<sub>2</sub> will disappear when a solution of KCN is added whereas SHb will still remain in the solution. Therefore, the calculation for SHb and HbO<sub>2</sub> levels needs a conversion factor which is calculated from  $A^{578} / A^{620}$  for normal blood. The results of the SHb levels from the 200 samples are shown in **Table 2**. There was no significant difference between males and females. The average (mean) and standard deviation (SD) of percentage of SHb was  $0.66 \pm 0.28$  % ranging from 0.02 to 1.10 %.

**Table 2** Percentage of Sulfhemoglobin (% SHb) in 200 Walailak University students.

Gender	N	SHb (%)		
		Mean $\pm$ SD	Min	Max
Male	100	0.66 $\pm$ 0.29	0.02	1.10
Female	100	0.66 $\pm$ 0.27	0.03	1.07
Total	200	0.66 $\pm$ 0.28	0.02	1.10

The MHb and SHb are 2 types of abnormal hemoglobin derivatives in the blood stream of the body. These types of Hb derivatives are unable to bind and carry oxygen to the tissues of the body. This makes inspiration insufficient and may cause central cyanosis. In severe case it may cause death if MHb is greater than 70 % [27]. There are very few reports of MHb and SHb levels in Thai people. The first study was reported by Dhareeruchata and co-workers [26] in 1997 in Bangkok, Thailand. Our results revealed that both MHb and SHb levels of students of Walailak University were rather lower than the previous study which reported levels of MHb of  $1.52 \pm 0.18$  % and levels of SHb of  $1.04 \pm 0.13$  %. However, both markers were in a normal range (MHb < 2 % and SHb < 1 %). Our results of MHb levels correlated to the study of Rodkey and O'Neal [22] which reported that MHb levels are typically about 1 - 3 % and results of SHb levels which best correlated to the study of Nichol and Morell [25] which reported SHb levels less than 1 %.

### CONCLUSIONS

Determination of MHb and SHb levels using spectrophotometry is an appropriate and suitable method since it is simple and easy to perform in a general routine laboratory. However it should be tested within 1 h after blood collection for good results (data not presented). Our results showed that MHb levels were  $1.29 \pm 0.57$  % and SHb levels were  $0.66 \pm 0.28$  % which no significant difference between males and females. The results correlated well with previous reports. Moreover, it revealed that both MHb and SHb levels of students in Walailak University were lower than the study of people in Bangkok which is a big city, very cloudy and has frequent traffic jams. It means that the surrounding environment at Walailak University is better than in Bangkok. Therefore, the measurement of the levels of MHb and SHb in peripheral blood can be used to monitor the degree of pollution in the environment.

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## บทคัดย่อ

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ระดับของเมทฮีโมโกลบินและซัลฟีฮีโมโกลบินในนักศึกษามหาวิทยาลัยวลัยลักษณ์

เมทฮีโมโกลบินและซัลฟีฮีโมโกลบินเป็นฮีโมโกลบินผิดปกติที่พบได้ในกระแสเลือด ใช้เป็นข้อมูลระบุความรุนแรงของมลพิษในสิ่งแวดล้อมรอบๆ ตัวเรา ได้แก่ อากาศ อาหาร และ น้ำ เมทฮีโมโกลบินเป็นรูปแบบออกซิไดส์ของฮีโมโกลบิน ที่เหล็กในส่วนของฮีโมโกลบินเปลี่ยนจากเฟอร์รัสเป็นเฟอร์ริกด้วยสารออกซิไดซิง ซึ่งส่วนใหญ่ได้รับจากทางอากาศ อาหาร และ ยา ส่วนซัลฟีฮีโมโกลบินเป็นรูปแบบการเติมกำมะถันเข้าไปในโมเลกุลของสายโพลีเปปไทด์ของฮีโมโกลบิน โดยการทำปฏิกิริยากับก๊าซไฮโดรเจนซัลไฟด์และก๊าซซัลเฟอร์ไดออกไซด์ที่ปนเปื้อนอยู่ในอากาศ หรือ ยาที่มีองค์ประกอบของกำมะถัน ฮีโมโกลบินทั้งสองชนิดนี้ไม่สามารถจับกับออกซิเจน ดังนั้นจึงอาจทำให้เกิดภาวะการขาดออกซิเจนได้ ถ้ามีในปริมาณมากเกินไปในกระแสเลือด วัตถุประสงค์ของการศึกษารั้งนี้เพื่อตรวจวัดระดับของเมทฮีโมโกลบินและซัลฟีฮีโมโกลบินในนักศึกษาที่พักอาศัยและเรียนอยู่ภายในมหาวิทยาลัยวลัยลักษณ์ โดยเก็บตัวอย่างเลือดครบส่วนจากนักศึกษาจำนวน 200 คน และใช้วิธีฮีโมโกลบินที่แยกแยะกันเลือดแข็ง จากนั้นวิเคราะห์หาปริมาณของเมทฮีโมโกลบินและซัลฟีฮีโมโกลบินด้วยวิธีสเปกโตรโฟโตเมตริ ผลที่ตรวจวัดได้รายงานเป็นค่าเฉลี่ยและค่าเบี่ยงเบนมาตรฐานของร้อยละของเมทฮีโมโกลบิน และร้อยละของซัลฟีฮีโมโกลบิน ในนักศึกษาจำนวน 200 คน คือ  $1.29 \pm 0.57$  เปอร์เซ็นต์ และ  $0.66 \pm 0.28$  เปอร์เซ็นต์ ตามลำดับ ระดับของเมทฮีโมโกลบินและซัลฟีฮีโมโกลบินในนักศึกษาของมหาวิทยาลัยวลัยลักษณ์ยังอยู่ในเกณฑ์ต่ำ เมื่อเปรียบเทียบกับข้อมูลของพลเมืองในเมืองใหญ่อย่างกรุงเทพมหานคร ซึ่งมีความแออัดมาก และมีสิ่งแวดล้อมที่เป็นมลพิษมากกว่าจากการจราจรที่หนาแน่น แสดงว่าสภาวะแวดล้อมโดยรวมมหาวิทยาลัยวลัยลักษณ์ยังคงสะอาด ดี และสดชื่นอยู่