

## Research article

# Screening of Thalassemia Carrier Traits in Students Using the One Tube Osmotic Fragility Method

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## Abstract.

Thalassemia is an inherited disorder resulting from a mutation or deletion of a gene that causes one of the globin chains to experience a decrease or cessation in the rate of synthesis. This leads to a decrease in the rate of haemoglobin synthesis. The highest prevalence of thalassemia in the world is found in tropical countries. The incidence of thalassemia carriers in Indonesia ranges from 6-10%. Thalassemia screening can be carried out by several examinations, including one tube osmotic fragility (OTOF) examination, red blood cell index, peripheral blood morphology and electrochemistry of haemoglobin. This study aimed to examine the screening of thalassemia carriers in undergraduate students using osmotic fragment examination of the OTOF method. This study used descriptive methods with 100 TLM D3 female students not yet known to have thalassemia. Univariate analyses were carried out. The results of the capillary blood samples indicated that 12% of the students were positive (indicating a suspected carrier of thalassemia) and 88% were negative according to the OTOF method using a hypotonic solution of 0.36% NaCl. From the positive OTOF results, the percentage of haemolysis was measured with varying results (17% - 65%). Those who had positive OTOF results in general were without significant symptoms. The findings accentuate that early detection of thalassemia is important.

**Keywords:** thalassemia, OTOF, microcytic, hypochromic

## 1. Introduction

Thalassemia disease is found worldwide with the highest prevalence of the gene in some tropical countries. Data from the World Bank shows that 7% of the world's population is a carrier of Thalassemia trait. Indonesia is one of the countries with a high frequency of Thalassemia carriers. This is evidenced by epidemiological studies in Indonesia which found that the frequency of the beta Thalassemia gene ranged from 3-10%[1].

Initial screening of this disorder to determine the suspect of Thalassemia before a definite diagnosis through genetic testing, a single tube osmotic fragility test (*One Tube Osmotic Fragility* / OTOF) can be performed, then a simple hematological examination

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in the form of Hb levels, red blood cell counts, MCV and Mentzer index has been determined, known and tested can determine the suspect of Thalassemia [2]. OTOF was the first method used to screen for Thalassemia and was introduced as a simple detector to detect Thalassemia trait carrier [3]. Students are a period of productive age that has the potential to give birth to offspring carrying Thalassemia traits so that the incidence of Thalassemia can be suppressed by conducting education and screening of Thalassemia carriers. This educational activity is very important to provide information and detect early potential for thalassemia, because certain types of thalassemia do not cause symptoms so it is difficult to detect. Based on the results of screening conducted Rosita et al in 2007 there are 29 subject (age 1-14 year) with 18% is major thalassemia and by Ayumnah P et al in 2016 on high school students, 15 % positive result were obtained, this informs that the OTOF methode has been carried out on different objects. In this study, the percentage of hemolysis was added by measuring Optical Density (OD) of the positive OTOF results using a spectrophotometer [2,4].

## 2. Methods

### 2.1. Desain Study

This research was conducted in February 2018. The population size was calculated based on a cross sectional survey, resulting in a total of 100 for the sample to be examined. These 100 samples were taken randomly from level 1 to level 3. The inclusion criteria for this study were female student who were willing to have their blood drawn as evidenced by giving informed consent and were not known to have Thalassemia.

### 2.2. Data Collection Prosedure

Counselling was conducted to recruit research subject and continued with explanation before the study ended with the distribution of informed consent to research subject. Respondents who signed informed consent underwent to laboratory examination by taking capillary blood. These research must adhere to the basic ethical principles : respect for persons, beneficence and justice. The method used in this research is descriptive which will only describe the results of screening for carriers of Thalassemia trait in 100 students of the D3 Medical Laboratory Technology study program using the OTOF method of osmotic fragility examination. The study began with giving informed

consent to the respondents, then taking specimens from capillaries and followed by examination of the osmotic fragility of the OTOF method.

### 2.3. Data Analysis

The data obtained comes from primary data from the OTOF examination, then the percentage is calculated with the following calculations:

$$x = \frac{\text{Number of OTOF positive result}}{\text{Total Respondent examined}} \times 100 \%$$

Examination of the percentage of hemolysis from positive OTOF results using a spectrophotometer, with the following calculations [4]:

$$\% \text{ Hemolysis} = \frac{\text{Sample Abs} - \text{positive control abs}}{\text{Abs negative control} - \text{positif control absorban}} \times 100\%$$

### 3. Results

In this study, 100 samples were collected from TLM D3 Stikes Achamd Yani Cimahi student. The results of the OTOF method of osmotic fragility examination obtained results as shown in table 1 below:

TABLE 1: One Tube Osmotic Fragility Result.

No	Result	Amount
1	Positive	12 %
2	Negative	88 %

The results of the examination of the percentage of hemolysis from positive OTOF results:

Positive control absorbance: 0,092

Negative control absorbance: 1,433

TABLE 2: Positive OTOF Haemolysis Percentage.

No	Sample code	Absorbance	% Haemolysis
1	AM	0,800	55
2 3 4	DN AZ F L DH	0,250 0,501 0,716	17 34 49 34 42 34 43
5 6 7	K AB IT HM CW	0,488 0,607 0,489	65 47 24 58
8 9	DM	0,692 0,933 0,678	
10 11		0,353 0,845	
12			

## 4. Discussion

Student of TLM D3 totally 120 student, 100 student participated in Thalassemia counselling, the explanation of this study and examination was carried out, 12 % was found to be positive for OTOF and from the positive OTOF results, the percentage of hemolysis was calculated, variation of hemolysis percentage is 17-58 %.

Based on the results of screening research for carriers of Thalassemia in students using OTOF examination of 100 samples, positive results were 12% and negative results were 88%. A positive OTOF result was caused by an abnormal sample of erythrocytes, where the size of the microcytic erythrocytes was hypochromic, allowing erythrocytes to have a wider surface size. Erythrocytes are able to accommodate more hypotonic solutions so that erythrocytes are able to accommodate more hypotonic solutions so that the erythrocytes are more resistant to hypotonic solutions. Erythrocytes do not undergo complete hemolysis and the remaining pieces of erythrocytes that are not hemolyzed will form erythrocyte deposits at the bottom of the tube. While a negative result is caused in a hypotonic solution the erythrocytes can absorb well the hypotonic solution and then complete hemolysis so that there are no erythrocyte deposits at the bottom of the tube and when the tube is placed on a paper background the writing will be clearly visible [5]. OTOF examination is influenced by the shape of erythrocytes, erythrocyte surface, volume and function of the erythrocyte membrane. In patients with thalassemia, there is a disturbance in the formation of the globin chain which causes the shape of the erythrocytes to vary and there are many hypochromic and microcytic erythrocytes found in the form of tear drop, target cells and ovalocytes [6]. Variations in the shape of erythrocytes affect the resistance of the erythrocyte membrane to the hypotonic solution where erythrocytes tend to be resistant to hypotonic solutions so that they do not undergo hemolysis. In this situation there is a decrease in osmotic fragility. Positive OTOF results indicate thalassemia, iron deficiency anemia and sickle cell anemia [7]. Limitations in this study has not been carried out with a definite diagnosis of people with suspected Thalassemia results, so it is highly recommended to perform haemoglobin electrophoresis or HPLC analysis as a follow-up examination in determining thalassemia.

## 5. Conclusions

Based on the results of screening research for carriers of Thalassemia traits from 100 samples of TLM D3 students using the OTOF method, it can be concluded that the

results of OTOF are positive 12%. This makes the role of early detection plays in a role in Thalassemia important in deciding the birth of people new Thalassemia.

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