RMJ Ramathibodi Medical Journal

Rama Med J | Original Article

# Thalassemia and Hemoglobinopathies Screening by Osmotic Fragility (OF) and Dichlorophenol Indophenol Precipitation (DCIP) Tests Among Vocational Students in Ubon Ratchathani Province

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**Background:** Thalassemia and hemoglobinopathies are the most common and clinically serious single gene disorders. Screening by osmotic fragility (OF) test and dichlorophenol indophenol precipitation (DCIP) test have been found to be effective and low-cost approaches to identify those who are carriers especially among young population.

**Objective:** To study the prevalence rates of abnormal of OF test and/or abnormal DCIP test among vocational students in Ubon Ratchathani province.

**Methods:** This cross-sectional research collected data from 311 students aged 15 to 19 years in a vocational school in Ubon Ratchathani, Thailand. OF and DCIP tests were done after participants signed consent to join the study. Knowledge and attitude towards thalassemia and thalassemia screening was obtained from self-administered questionnaires. Chi-square and Fisher exact tests were performed to examine the association between variables.

**Results:** Of 311 students, 124 (39.9%) students had abnormal OF test or DCIP test or both, 72 (23.2%) students had both DCIP and OF tests positive. The final diagnosis was that 75.8% had hemoglobin E, 21.8% and 2.4% were α-thalassemia and β-thalassemia carriers respectively. Approximately, 91.4% of them considered thalassemia screening useful and necessary for premarital screening.

**Conclusions:** High incidence of abnormal OF test and/or DCIP test was found among vocational students in Ubon Ratchathani province, showing a predominance of hemoglobin E. Most of them agreed that thalassemia screening is useful for adolescents to avoid marriage among carriers.

**Keywords:** Thalassemia screening, OF test, DCIP test, Vocational students, Hemoglobin E

**Rama Med J:** doi:10.33165/rmj.2021.44.2.249723

**Received:** April 12, 2021 **Revised:** May 11, 2021 **Accepted:** June 18, 2021

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## Introduction

Hemoglobin (Hb) disorders are some of the most common and clinically serious single gene disorders. It is estimated that about 250 million people (4.5% of the world population) carry a potential pathological hemoglobinopathies gene and about 300 000 infants are born with major hemoglobinopathies each year.[1] The present management of the disease gives affected persons a prolonged life expectancy beyond the third or fourth decade. Quality of life for patients and their families represents public health services, which clearly underline the fundamental aspect of prevention rather than treatment.[2]

Thalassemia is distributed around the world, including across Africa, the Mediterranean region, the Middle East, the Indian subcontinent, China and throughout Southeast Asia in a line stretching from Southern China down the Malaysian peninsula to the Indonesian islands.[3, 4] Among these populations, the carrier frequency is greater than 1%, in contrast to a carrier frequency of approximately 0.1% in individuals of Northern European ancestry. Sickle cell disorders are seen more frequently in populations of African and Caribbean descent and in individuals of Mediterranean, Middle Eastern, and East Indian descent.[5, 6] The prevention of thalassemia by combining 4 strategies through education, carrier screening, counseling and prenatal diagnosis has proved to be effective, acceptable and highly cost-beneficial.[7]

Thalassemia carrier screening is the most widely performed genetic screening test in many different countries. β-thalassemia carrier screening programs provide a unique opportunity to compare the delivery of carrier screening programs carried out in different cultural, religious and social contexts.[8] Carrier screening programs aim to identify asymptomatic carriers of recessive conditions so that they are informed and understand their reproductive risks and options.[9-11] National and international guidelines recommended that genetic screening should not be compulsory for any individual or population. They also recommended that appropriate information be provided to individuals before testing to enable informed decision making about genetic screening and that genetic screening be accompanied by counseling.[12, 13] Hemoglobinopathies screening programs by hemoglobin typing are offered on a voluntary basis. In Canada, voluntary programs have been conducted in high schools, as well as before pregnancy and antenatally for more than 20 years, which reduced the incidence of diseases by 90% to 95%.[14] In Thailand, screening of thalassemia has been conducted only in premarital or preconceptional situations. It has never been done among adolescents or in high school students because it is considered to be very expensive and may not be as effective since adolescents may not use this information later in life.

In Thailand, the frequency of α-thalassemia reaches 20% to 30% in Bangkok and northern Thailand, while the frequency of β-thalassemia varies between 3% to 9%. The incidence of thalassemia and hemoglobinopathies in the Thai population reaches 32.5%.[15] The aim of the Thai National Health policy is to prevent severe forms of thalassemia, including Hb Bart's hydrops fetalis, homozygous β-thalassemia and β-thalassemia/Hb E (Hemoglobin E). To achieve this, a combination of osmotic fragility (OF) and dichlorophenol indophenol precipitation (DCIP) tests have been used in mass screening among married couples or pregnant women. OF test is sensitive enough to detect almost all cases of α-thalassemia and β-thalassemia carriers,[16] whereas the blue dye DCIP test can be used as a screening test for Hb E. A combination of OF and DCIP has been shown to be a reliable, rapid, simple, inexpensive and sensitive strategy for screening thalassemia and Hb E in pregnant Thai women.[17]

Ubon Ratchathani province is located in the northeast region of Thailand, where thalassemia and hemoglobinopathies are common (30% - 40%). This study aimed to explore the feasibility of applying OF and DCIP test in screening thalassemia and hemoglobinopathies among adolescents in a vocational school and to explore the level of knowledge and attitude towards thalassemia and thalassemia screening.

## Methods

Approval for the study was obtained from the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand, No. MURA2013/481 on September17, 2013.

This cross-sectional descriptive survey research recruited 311 volunteer students from a vocational school in Ubon Ratchathani province. Data was collected by self-administered questionnaires. The reliability of the questions concerning knowledge and attitude towards thalassemia and thalassemia screenings was determined by calculating Cronbach's alpha coefficients in a pilot study of 30 adolescents in another comparable vocational school. The coefficients were 0.78 and 0.82 for knowledge and attitude, respectively. The validity of the questionnaires was determined by 3 experts, who evaluated and improved the questionnaires.

The population comprised students in a vocational school aged between 15 to 19 years. They could read and write Thai and were willing to cooperate with the study. Sample size was calculated by Daniel's formula[18] using the prevalence of thalassemia or abnormal hemoglobin in Thailand, which was 40%.[19]

Self-administered questionnaires were constructed according to the objectives of the study and were divided into 3 parts (general characteristics, results of OF and DCIP tests, and knowledge and attitude towards thalassemia and thalassemia screening). Students who had abnormal OF or DCIP tests were subsequently investigated by hemoglobin typing, which was done at the thalassemia hematological laboratory of Regional Health Promotion Center 10. The results of hemoglobin typing were interpreted by an obstetrician and an internist at the Regional Health Promotion Center 10.

The questionnaires contained 12 questions about knowledge of thalassemia and thalassemia screening. Correct answers for each question gained 1 point. Mean and standard deviation (SD) scores of knowledge were 5.58 and 1.77, respectively. Level of knowledge was classified into 2 levels as poor and good using the cutoff score of 8. Eight points or more were classified as good knowledge.

Attitude towards thalassemia and thalassemia screening was evaluated by scoring 5 levels of agreement (strongly agree, agree, uncertain, disagree, and strongly disagree). There were 12 questions of attitude (11 positive questions and 1 negative question). The scoring for each question was done according to the level of agreement. For positive questions, the scoring was 5, 4, 3, 2, and 1 accordingly to level of agreement; strongly agree, agree, uncertain, disagree, and strongly disagree, respectively. For negative questions, the scoring was reversed. Mean and SD score of attitude were 41.26 and 3.98, respectively. Level of attitude was classified into 2 levels as poor and good using the cutoff scores of 45. Score of 45 or more was classified as good attitude.

SPSS software package version 18 (PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc; 2009) licensed to Mahidol University was used for data analysis. Chi-square and Fisher exact tests were applied to test the association between these variables with a significance level at $P < .05$.

## Results

Among of 311 students, 186 students (59.8%) were female and 210 (50.2%) were aged 18 to 19 years, mean (SD) age was 18.62 (1.03) years, 79.4% studied at the diploma level, 87.1% were born in Ubon Ratchathani province, and 84.9% had monthly family income less than ฿20 000. Most students (98.4%) had no history of thalassemia or hemoglobinopathies, only 5 students (1.6%) reporting that they had thalassemia or hemoglobinopathies. Also, most students (97.4%) had no family history of thalassemia or hemoglobinopathies, only 8 students (2.6%) reported that they had a family history of thalassemia and hemoglobinopathies (Table 1).

Diagnosis of thalassemia or hemoglobinopathies among students showed that 124 (39.9%) students had abnormal OF or DCIP, or both, 72 (23.2%) students had both DCIP and OF positive, 23 (7.4%) students had only DCIP positive, and

29 (9.3%) students had only OF positive. When hemoglobin typing was applied, Hb E (heterozygous or homozygous) was found in 75.8% , α-thalassemia carrier was found in 21.8%, and only 2.4% of β-thalassemia carrier was found (Table 2).

Most vocational students (272 cases or 87.5%) had a poor level of knowledge about thalassemia and thalassemia screening (Table 3). Female students had a significant higher level of knowledge than males ($P < .001$). Students at the diploma level had better knowledge than students at the certificate level ($P < .05$). Most of students (83.9%) had a poor attitude towards thalassemia and thalassemia screening. Female students had a significantly better level of attitude than males ($P < .001$). Students aged less than 19 years had a significantly better level of attitude than older students ($P < .01$) (Table 3).

Questions which had a high percentage of correct answers were that blood testing is the only way to diagnose thalassemia (86.8%), that thalassemia trait is genetically transmitted (72.0%), and that bone marrow transplant is the only way to cure thalassemia disease (63.7%). Questions which got a low percentage of correct answers included the knowledge about geographic distribution of thalassemia in Thailand (9.6%), and probability of newborns having thalassemia disease when both parents have thalassemia trait (13.8%) (Table 4).

Item of attitude with the highest agreement among answers was that premarital or preconception thalassemia screening helps to prevent and control the incidence of thalassemia disease. Two items of attitude with the least agreement were that no screening was needed if they and their family had no history or signs or symptoms and that termination was an option because if the fetus had severe thalassemia disease (Table 5). Students who responded that they had a history or family history of thalassemia did not have significantly better knowledge or better outlook towards thalassemia or thalassemia screening (Table 4 and 5).

**Table 1. Characteristics of the Vocational Students**

| Characteristic | No. (%) |
|---|---|
| Gender | |
| Male | 125 (40.2) |
| Female | 186 (59.8) |
| Age, y | |
| ≤ 18 | 155 (49.8) |
| > 18 | 156 (50.2) |
| Education | |
| Certificate | 64 (20.6) |
| Diploma | 247 (79.4) |
| Monthly family income, ฿ | |
| ≤ 20 000 | 264 (84.9) |
| > 20 000 | 47 (15.1) |
| Birthplace | |
| Ubon Ratchathani | 271 (87.1) |
| Other province | 40 (12.9) |
| History of thalassemia and hemoglobinopathies | |
| Yes | 5 (1.6) |
| No | 306 (98.4) |
| Family history of thalassemia and hemoglobinopathies | |
| Yes | 8 (2.6) |
| No | 303 (97.4) |

**Table 2. Diagnosis of Thalassemia or Hemoglobinopathies Among Students With Abnormal DCIP and OF Test**

| Test Results | Hemoglobin Typing, No. (%) | | | | |
|---|---|---|---|---|---|
| | Total (N = 311) | EA | EE | β | α |
| Normal | 187 (60.1) | - | - | - | - |
| Abnormal | 124 (39.9) | 79 (63.6) | 15 (12.1) | 3 (2.4) | 27 (21.8) |
| DCIP positive | 23 (7.4) | 23 (18.5) | - | - | - |
| OF positive | 29 (9.3) | - | - | 3 (2.4) | 26 (21.0) |
| DCIP positive with OF positive | 72 (23.2) | 56 (45.1) | 15 (12.1) | - | 1 (0.8) |

Abbreviations: α, alpha-globin gene; β, beta-globin gene; DCIP, dichlorophenol indophenols precipitation; EA, heterozygous hemoglobin E; EE, homozygous hemoglobin E; OF, osmotic fragility.

**Table 3. Association Between Characteristics of Students and Level of Knowledge and Attitude Towards Thalassemia and Thalassemia Screening**

| Characteristic | Level of Knowledge, No. (%) | | *P* Value* | Level of Attitude, No. (%) | | *P* Value* |
|---|---|---|---|---|---|---|
| | Poor Score 1 - 7 | Good Score 8 - 12 | | Poor Score 1 - 45 | Good Score 46 - 60 | |
| Total (N = 311) | 272 (87.5) | (39) (12.5) | | 261 (83.9) | 50 (16.1) | - |
| Gender | | | | | | |
| Male | 118 (94.4) | 7 (5.6) | < .001* | 112 (89.6) | 13 (10.4) | < .001* |
| Female | 154 (82.8) | 32 (17.2) | | 149 (80.1) | 37 (19.9) | |
| Age, y | | | | | | |
| < 19 | 131 (84.5) | 24 (15.5) | .16 | 122 (78.7) | 33 (21.3) | .04* |
| ≥ 19 | 87 (85.4) | 15 (9.6) | | 139 (89.1) | 17 (10.9) | |
| Education | | | | | | |
| Certificate | 52 (81.2) | 12 (18.8) | .01* | 50 (78.1) | 14 (21.9) | .27 |
| Diploma | 220 (89.1) | 27 (10.9) | | 236 (85.4) | 36 (14.6) | |
| Monthly family income, ฿ | | | | | | |
| ≤ 20 000 | 116 (85.9) | 19 (14.1) | .29 | 110 (81.5) | 25 (18.5) | .56 |
| > 20 000 | 45 (95.7) | 2 (4.3) | | 151 (85.8) | 25 (14.2) | |
| Birthplace | | | | | | |
| Ubon Ratchathani | 237 (87.5) | 34 (12.5) | .34 | 237 (87.5) | 34 (12.5) | .34 |
| Other province | 35 (87.5) | 5 (12.5) | | 35 (87.5) | 5 (12.5) | |
| History of thalassemia and hemoglobinopathies | | | | | | |
| Yes | 4 (80.0) | 1 (20.0) | .49[b] | 5 (100.0) | 0 | 1.00[b] |
| No | 268 (87.6) | 38 (12.4) | | 256 (83.7) | 50 (16.3) | |
| Family history of thalassemia and hemoglobinopathies | | | | | | |
| Yes | 6 (75.0) | 2 (25.0) | .26[b] | 4 (80.0) | 1 (20.0) | .12[b] |
| No | 266 (87.8) | 37 (12.2) | | 268 (87.6) | 38 (12.4) | |

* Statistical significance level at $P < .05$.
[a] Chi-square test.
[b] Fisher exact test.

**Table 4. Number and Percent of Correct Answers in Questions About Thalassemia and Thalassemia Screening**

| Item | No. (%) of Correct Answers (N = 311) |
|---|---|
| Blood testing is the only way to diagnose thalassemia. | 270 (86.8) |
| Thalassemia traits can be genetically transmitted. | 224 (72.0) |
| Bone marrow transplant is the only way to cure thalassemia disease in addition to blood transfusion and chelating treatment. | 198 (63.7) |
| The cost of treatment for thalassemia disease is as high as ฿180 000 to ฿250 000 per year. | 192 (61.7) |
| Signs and symptoms of thalassemia include: fatigue, weakness, pale or yellowish skin, facial bone deformities, slow growth and abdominal swelling due to hepatosplenomegaly. | 188 (60.5) |

**Table 4. Number and Percent of Correct Answers in Questions About Thalassemia and Thalassemia Screening (Continued)**

| Item | No. (%) of Correct Answers (N = 311) |
|---|---|
| To prevent the occurrence of thalassemia disease, screening among adolescents is the most appropriate approach. | 147 (47.3) |
| Thalassemia is a disease of abnormal red blood cells. | 140 (45.0) |
| The probability of a newborn being a thalassemia carrier is 50% if one parent has a homozygous gene and one parent is normal. | 114 (36.7) |
| The probability of a newborn having thalassemia disease is 50% if one parent has the disease and one parent is a carrier. | 103 (33.1) |
| Thalassemia carriers look healthy and normal, but can transmit the thalassemia gene to their offspring. | 89 (28.6) |
| The probability of a newborn having thalassemia disease is 25% if the mother and father are carriers of the same thalassemia trait. | 43 (13.8) |
| High geographic distribution of thalassemia in the north and northeast region of Thailand. | 30 (9.6) |

**Table 5. Attitude Towards Thalassemia and Thalassemia Screening**

| Item | Mean (SD), % of Attitude* (N = 311) |
|---|---|
| Premarital or preconception thalassemia screening help prevent and control the incidence of thalassemia disease. | 4.57 (0.53), 91.4 |
| If both members of a married couple are carriers of thalassemia genes, they should consult with a doctor before getting pregnant. | 4.52 (0.66), 90.4 |
| Healthy men and women should have thalassemia screening before marriage and pregnancy. | 4.48 (0.60), 89.6 |
| Both men and women of reproductive age should receive thalassemia screening. | 4.36 (0.70), 87.2 |
| Thalassemia screening reduces the risk of thalassemia diseases in offspring. | 4.35 (0.76), 87.0 |
| Thalassemia screening with genetic counseling in couples who are at risk of thalassemia in offspring help couples to choose the best options. | 4.29 (0.76), 85.8 |
| Thalassemia screening is the preliminary step. If abnormality is found, further testing should be done. | 4.24 (0.64), 84.8 |
| Carriers of the thalassemia gene should tell their boyfriends or girlfriends. | 4.22 (0.83), 84.4 |
| Thalassemia screening has some costs, but it is beneficial to know about any abnormality. | 4.22 (0.64), 84.4 |
| Lifelong palliative treatment of thalassemia affects the patient's life and family. | 4.05 (0.90), 81.0 |
| Termination of pregnancy is an option for a fetus with severe thalassemia disease. | 3.86 (1.04), 77.2 |
| There is no need for thalassemia screening in a family with no history of thalassemia or hemoglobinopathies. | 2.50 (1.28), 50.0 |

Abbreviation: SD, standard deviation.

* Level of attitude towards thalassemia and thalassemia screening varied from 1 (minimum) to 5 (maximum).

## Discussion

Inherited hemoglobin disorders are an important problem in many developing countries including Thailand. Although a Thai national policy to antenatally screen couples at risk of having affected babies with severe thalassemia has been implemented for decades, at least 12 000 patients are born each year.[20] An important reason for these preventable events is late first antenatal visit. It was recommended that thalassemia screening be used[14] starting from early child-bearing age. In Canada, 4 processes and outcome variables were reported; 1) voluntary participation rates in a high-school cohort, 2) uptake rates for the screening test, 3) origin of carrier couples seeking the prenatal diagnosis option in the programs, and 4) change in incidences of the diseases. Using these strategies, incidence of 2 diseases fell by 90% to 95% over 2 decades.[14]

The aim of carrier screening is to identify the carriers of hemoglobin disorders in order to assess the risk for a couple having a severely affected child and to provide information on the options available to avoid such an occurrence. A screening method by using OF and DCIP test is in agreement with the concept of present health economics as the cost of this method is much lower than the standard hemoglobin electrophoresis method.[21]

This is the first thalassemia screening study conducted among adolescents in Thailand. The screening of adolescents is a simple way to prevent marriage among carriers, though the topic is still controversial in Thailand because of inadequate funding.

The results of hemoglobin typing showed that Hb E (heterozygous or homozygous) was found in 30.2%, which was similar to the study of Bunthupanich et al,[22] who studied thalassemia and hemoglobinopathies among Laos, Khmer, Suay and Yer ethnic groups residing in lower Northeastern Thailand. They demonstrated an extremely high prevalence of Hb E.

The cost of screening for thalassemia using a combination of OF test and DCIP test was not expensive. OF test costs about ฿50 per case, while DCIP test costs about ฿70 per case. Such costs are affordable when compared to the cost of screening by hemoglobin typing and cost of treatment for a thalassemia patient. This is in agreement with the study of Ahmadnezhad,[23] who found that the cost of a screening and prenatal diagnosis program was much lower than the cost of treatment in potential thalassemia patients. Screening should start during early childhood or adolescence.

Female students had better knowledge than male students, which was in accordance with Miri-Moghaddam et al,[24] who studied high school knowledge and attitudes towards thalassemia in Southeastern Iran.

The significant higher knowledge level among the certificate group student than the diploma one can be explained by the significant higher female composition in the certificate group (78.1%) compare to only 55.1% among diploma group ($P < .01$).

Programs providing knowledge and proper perspective should also be established in the region because this study showed that students who were known to have a history of thalassemia or family history of thalassemia did not show significantly better knowledge and attitude towards thalassemia or thalassemia screening. This may be because they had lack of or incorrect knowledge of thalassemia.

## Conclusions

The combination of OF and DCIP tests is cheap and effective to screen thalassemia among adolescents. In this study, high incidence of abnormal OF and DCIP were found among vocational students with predominance of hemoglobin E. Most of them agreed that thalassemia screening would be useful and could be used to avoid offspring between carriers.

## Acknowledgements

This study was partially supported by the Ramathibodi Annual Research Fund (2013), Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand.

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# การตรวจคัดกรองธาลัสซีเมียและฮีโมโกลบินผิดปกติ โดยวิธี Osmotic Fragility (OF) และ Dichlorophenol Indophenol Precipitation (DCIP) ในนักเรียนอาชีวศึกษา ในจังหวัดอุบลราชธานี

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**บทนำ:** ธาลัสซีเมียและความผิดปกติของฮีโมโกลบินเป็นโรคเลือดทางพันธุกรรมจากยีนเดี่ยวที่ร้ายแรงและพบบ่อยที่สุด การตรวจกรองโดยวิธี Osmotic fragility (OF) และวิธี Dichlorophenol indophenol precipitation (DCIP) พบว่ามีประสิทธิภาพสูงในการค้นหาผู้ที่เป็นพาหะและราคาไม่แพง เหมาะสำหรับการคัดกรองในวัยรุ่น

**วัตถุประสงค์:** เพื่อศึกษาความชุกของความผิดปกติที่พบจากการตรวจคัดกรองธาลัสซีเมียโดยวิธี OF และ DCIP ในนักเรียนโรงเรียนอาชีวศึกษาแห่งหนึ่งในจังหวัดอุบลราชธานี

**วิธีการศึกษา:** การศึกษาวิจัยเชิงพรรณนาแบบภาคตัดขวางของข้อมูลในกลุ่มตัวอย่างนักเรียนวิทยาลัยอาชีวศึกษาแห่งหนึ่งในจังหวัดอุบลราชธานี ประเทศไทย จำนวน 311 คน อายุ 18 ถึง 19 ปี ได้รับการตรวจเลือดโดยวิธี OF และ DCIP และตอบแบบสอบถามเกี่ยวกับความรู้และทัศนคติต่อโรคธาลัสซีเมียและการตรวจคัดกรองธาลัสซีเมีย การวิเคราะห์ความสัมพันธ์ระหว่างตัวแปรใช้สถิติ Chi-square test และ Fisher exact test

**ผลการศึกษา:** จากกลุ่มตัวอย่างนักเรียน จำนวน 311 คน พบว่า นักเรียน 124 คน (ร้อยละ 39.9) มีผลตรวจ OF หรือ DCIP หรือทั้ง 2 อย่างผิดปกติ และนักเรียน 72 คน (ร้อยละ 23.2) มีผลผิดปกติทั้ง 2 อย่าง ผลการวินิจฉัยสุดท้ายพบว่า นักเรียนร้อยละ 75.8 เป็นฮีโมโกลบินอี ร้อยละ 21.8 เป็นพาหะแอลฟ่าธาลัสซีเมีย และร้อยละ 2.4 เป็นพาหะเบต้าธาลัสซีเมีย โดยกลุ่มตัวอย่างร้อยละ 91.4 มีความเห็นว่าการตรวจคัดกรองมีประโยชน์ที่จะนำไปตรวจคัดกรองก่อนสมรส

**สรุป:** การตรวจคัดกรองธาลัสซีเมียโดยวิธี OF และวิธี DCIP ในนักเรียนอาชีวศึกษาพบว่ามีความผิดปกติสูง ส่วนใหญ่เป็นฮีโมโกลบินอี และนักเรียนส่วนใหญ่เห็นว่าการตรวจคัดกรองธาลัสซีเมียมีประโยชน์สามารถนำไปใช้ในการเลือกคู่ครองเพื่อหลีกเลี่ยงการแต่งงานระหว่างคนที่เป็นพาหะธาลัสซีเมีย

**คำสำคัญ:** การตรวจคัดกรองธาลัสซีเมีย วิธี OF วิธี DCIP นักเรียนอาชีวศึกษา ฮีโมโกลบินอี

**Rama Med J:** doi:10.33165/rmj.2021.44.2.249723

**Received:** April 12, 2021 **Revised:** May 11, 2021 **Accepted:** June 18, 2021

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