Sensitivity and Specificity of Mean Corpuscular Hemoglobin (MCH): For Screening Alpha-thalassemia-1 Trait and Beta-thalassemia Trait

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Objective: *To evaluate sensitivity, specificity, and positive and negative predictive value of mean corpuscular hemoglobin (MCH) for screening alpha-thalassemia-1 trait and beta-thalassemia trait*
Material and Method: *Descriptive analysis, diagnostic test, was conducted on 396 pregnant women attending the antenatal clinic between September 2007 and June 2008. Blood samples were collected from pregnant women after counseling and getting informed consent. MCH was measured in all samples by automated hematology analyzer. Determination of HbA2 level for diagnosis of beta-thalassmia trait and PCR for alpha-thalassemia-1 gene (SEA type) were performed in all cases as a gold standard. The data were collected and analyzed for sensitivity, specificity, and positive and negative predictive value of MCH for screening alpha-thalassemia-1 trait and beta-thalassemia trait.*
Results: *Based on the ROC curve, the best cut-off level of MCH in predicting the thalassemia carriers was 26.5 picrograms. Positive MCH (< 26.5 picrograms) gave the sensitivity of 95.2% and specificity of 82.3% in screening alpha-thalassemia-1 trait and beta-thalassemia trait. The positive predictive value and negative predictive value were 40.4% and 99.3% respectively.*
Conclusion: *MCH is a good tool for screening alpha-thalassemia-1 trait and beta-thalassemia trait during pregnancy because of its simplicity, low cost, (when determined as a part of complete blood count), and high sensitivity.*

Keywords: *Erythrocyte indices, Alpha-Thalassemia, Beta-Thalassemia*

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Thalassemia is the most common hematologic genetic disease in Thailand. The high prevalence of α-thal1, β-thalassemia, and HbE gene in the Thai population, 14%, 3-9%, and 13% respectively[1,2], leads to many births of children with severe thalassemia, homozygous b-thalassemia, b-thalassemia/HbE, and Hb Bart's disease. The affected persons of the first two entities have a low quality of life and have estimated average life expectancy of 10 and 30 years, respectively. Hb Bart's hydropic fetuses have never survived and their mothers often suffer from obstetric complications such as pre-eclampsia, dystocia, post-partum hemorrhage due to a large placenta, and the psychological burden for carrying a nonviable fetus to term. Each year, the authors' department faces about 20 new cases of homozygous β-thalassemia, 30-40 new cases of β-thalassemia/HbE, and 20-30 new cases of Hb Bart's disease. Therefore, these three entities of severe thalassemia need to be controlled, especially by prenatal approach[3]. Recently, the authors have had great success in the control of severe thalassemia using a simple way that cost much less than that of fetal DNA analysis[4]. However, this screening system has significant false positive test, leading to unnecessary confirmatory tests. In Thailand, the algorithm of prenatal control of severe thalassemia is diversified. For example, there are several screening tests for alpha/beta thalassemia

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such as OFT, MCV, MCH etc. Several screening tests for Hb E are available such as DCIP, KKU-DCIP, or CMU-HbE screen test. MCH is one of the screening tests for thalassemia (alpha-thalassemia1 trait and beta-thalassemia). It has not been thoroughly evaluated for its efficacy in spite of the fact that this is a simple technique and is available on an automated machine determining complete blood count in routine practice. Although, some studies showed that this might be effective in screening a thalassemia carrier, using the cut-off point at < 26-28 fl[5-7], it has never been evaluated for the best cut-off level of MCH to discriminate normal and abnormal test.

The main purpose of the present study was to evaluate the efficacy of MCH measurement in screening α-thalassemia1 and β-thalassemia carriers, using HbA2 levels measured by microcolumn chromatography and PCR for α-thalassemia1 (SEA type) as a gold standard.

Material and Method

Pregnant women attending the antenatal care clinic at Maharaj Nakorn Chiang Mai Hospital between September 2007 and June 2008 were recruited into the present study. Ethical approval was given. The inclusion criteria consisted of 1) present in the first half of pregnancies, 2) no known thalassemia carrier (history of previous child with the disease, or previous screening in the previous pregnancy), and 3) not anemic. Exclusion criteria included women with loss to follow-up or the data on final outcomes could not be obtained. All of these women were counseled and invited to join the study with informed consent. The definition used in the present study included: 1) α-thalassemia-1 carrier: Individuals who has deletion of both loci from one chromosome (--/αα). In most populations, the α-globin chain cluster or gene loci are duplicated on chromosome 16. Thus, normal genotype for diploid cells can be expressed as αα/αα. A deletion of two genes (--/αα) results clinically in α-thalassemia 1 trait, which is characterized by minimal hypochromic microcytic anemia, usually not associated with clinical abnormality and often goes unrecognized. 2) β-thalassemia carrier: Individuals who have heterozygous state of β-thalassemia gene mutation, located on chromosome 11. With beta-thalassemia carrier state, hemoglobin A2, which is composed of two α- and two δ-globin chains, is increased to more than 3.5-4%. They are usually non-anemic.

The pregnant women recruited into the present study were taken care of as a standard antenatal care. Five milliliters of blood sample was taken for MCH test as a screening test for alpha-thalassemia1 or beta-thalassemia gene carriers, HbA2 level for diagnostic test of beta-thalassemia carrier, and Polymerase chain reaction (PCR; SEA type) as a gold standard in establishing the carrier status of alpha-thalassemia1. The laboratory techniques used in the present study included: 1) Mean corpuscular hemoglobin (MCH): MCH is defined by the mean corpuscular hemoglobin measured by automated hematology analyzer (Coulter STKS analyzer; Beckman, USA) and expressed picograms. The measurement is done on a blood sample of 1.0 ml in a test tube using EDTA as an anticoagulant MCH of less than 27 pgs is considered positive or abnormal. 2) PCR for α-thal-1 (SEA type): PCR in this project is used as a definite test for detection α-thal1 gene carrier. This is a technique for amplification and analysis of DNA of α-thal1 gene, modified from Chang's method (8;9) by changing the primer specific for α-thal-1 trait. In a normal subject, PCR product will consist of only 314 base pair type, but there are PCR products of 314 and 188 base pair types in blood sample from α-thal-1 trait. 3) Hb A2 test: A simple quantitative test (using the standard A2 column kit; microcolumn chromatography) is used to identify beta-thalassemia trait. The levels of normal, β-thal trait, HbE trait, and homozygous HbE of < 4%, 4-9%, > 10%, and > 60%, respectively (HbA2 and HbE appear in the same location of the column). Used as a diagnostic test for β-trait, or HbE trait.

The main outcome was a sensitivity, specificity, and positive and negative predictive value of MCH in predicting alpha or beta thalassemia trait, using HbA2 level and PCR for α-thalassemia 1 as a gold standard.

Based on previous studies, the present study needed a sample size of at least 300 pregnant women to gain power of test 80% with confidence interval of 95%, when the sensitivity of test was more than 97% and specificity of more than 75%.

Statistical analysis included 1) demographic data or obstetric background were presented as percentage, means and standard deviation etc. 2) accuracy of the screening tests was presented as detection rate (sensitivity), and specificity with 95% confidence interval (95% CI) in detecting thalassemia carrier status.

Results

During the present study, 396 pregnant women were recruited into the present study.

Twenty-one women were excluded from the present study because of incomplete data. The remaining 375 cases were available for analysis. The majority of the subjects resided in Chiang Mai province (80.8%). Most of them were between 21-30 years old (54.5%). The occupations were mostly employee (52.2%) and housewife (27.1%). Most (54.6%) were nulliparous.

Three hundred and seventy-five blood samples were sent to measure MCH by automated hematology analyzer and all blood samples were also sent to detect beta-thalassemia trait by HbA2 test (HbA2 4.1-9%) and alpha-thalassemia-1 trait by PCR for alpha- thalassemia-1 gene (SEA type). Beta-thalassemia trait or alpha- thalassemia-1 trait was diagnosed in42 samples.

Based on ROC curve (Fig. 1), the best cut-off level of MCH in predicting the thalassemia carriers was 26.5 picrograms. Positive MCH ($\leq$ 26.5 picrograms) gave the sensitivity of 95.2% and specificity of 83.9% in screening alpha-thalassemia-1 trait and beta-thalassemia trait. The positive predictive value was 37.9% and the negative predictive value was 99.1% (Table 1).

The positive screening test or MCH of $\leq$ 26.5 picograms was found in 99 samples and a negative result or MCH of more than 26.5 picograms in 276 samples. The mean ($\pm$ SD) hemoglobin concentration was 12.0 $\pm$ 1.2 gm/dl. The mean ($\pm$ SD) of MCH was 28.0 $\pm$ 3.3 picograms.

Of 375 pregnant women, there were 47 cases of Hb E trait (without alpha-thalassemia1 or beta carrier), which were also defined as negative final diagnosis or no thalassemia carrier. Twenty-four (55.3%) of them had a positive MCH test ($\leq$ 26.5 picograms) and 44.7% had a negative MCH test).

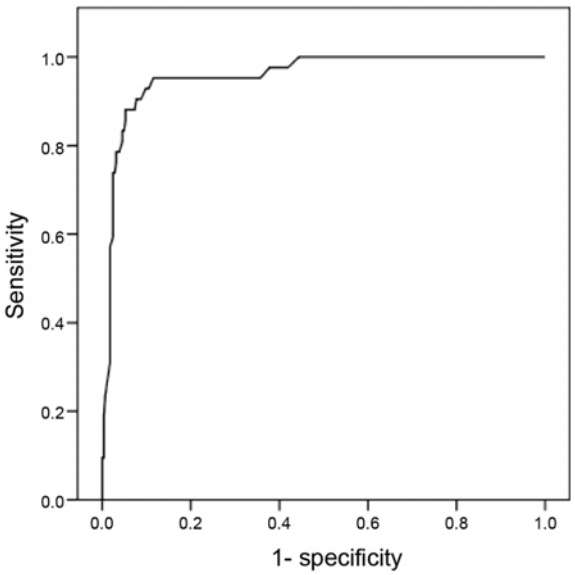

Fig. 1 ROC curve of MCH in predicting α-thalassemia-1 trait/β-thalassemia trait (area under curve 0.943)

Table 1. Two by two table shows the diagnostic indices of MCH in predicting alpha-thalalassemia1 trait or beta-thalassemia trait among non-anemic pregnant women

	α-thalassemia-1/ β-thalassemia carrier		Total
	Non-carrier	Carrier	0
MCH Negative	274	2	276
Positive	59	40	99
Total	333	42	375

Sensitivity: 95.2% (40/42) 95% [95% CI: 0.888-1.017]
Specificity: 82.3% (274/333) [95% CI: 0.782-0.864]
Positive predictive accuracy: 40.4% (40/99) [95% CI: 0.307-0.501]
Negative predictive accuracy: 99.3% (274/276) [95% CI: 0.976-1.009]

Discussion

Currently, widely used screening tests of beta-thalassemia or alpha-thalassemia-1 trait in the prevalent areas include mean corpuscular volume (MCV) and erythrocyte osmotic fragility test (EOFT). Both methods are advocated to have high sensitivity and specificity[10-12]. Maharaj Nakorn Chiang Mai Hospital used EOFT (0.45% glycerine saline solution) as a screening test of beta-thalassemia and alpha-thalassemia-1 trait[10]. However, the accuracy of MCH in screening thalassemia trait in pregnant women has not been studied. MCH could be easily screened at the first antenatal visit, using automated hematology analyzer together with complete blood count. Usually, this machine can report MCH concentration at the same time as routine complete blood count. Exclusion criteria in the present study were pregnancies that already had anemia or twins pregnancy. This was done to minimize confounding factors that may interfere with the result because many types of anemia have a direct effect on RBC volume and MCH measurement[13,14]. Moreover, in real practice, any case of anemia will completely be worked up to identify the causes including thalassemia. Therefore, there would not be any need for screening test.

Normal range of HbA2 level in the general literature was 2.5-3.5%, but it can be varied according to laboratories. In the present study, the authors used cutoff point of HbA2 level at 4%, which is the cutoff level at Maharaj Nakorn Chiang Mai Hospital Central Diagnostic Laboratory[10]. Our laboratory previously tested 128 known cases of beta-thalassemia trait that were the parents of homozygous beta-thalassemia patients and found that all of them had HbA2 > 4%, the majority of the cases ranged between 4.1-9%.

The data presented here showed that MCH at the cut-off level of ≤ 26.5 fl is a very good screening test for both beta-thalassemia trait and alpha-thalassemia-1 trait among asymptomatic pregnant women, giving a sensitivity of 95.2%, specificity of 82.3%, and negative predictive value of 99.3%. Interestingly, both false negative results in the present study were alpha-thalassemia-1 trait, indicating that MCH can screen all beta-thalassemia traits. However, positive predictive value was rather low leading to the need of the confirmatory diagnostic test in a relatively large number of women. Therefore, combination with other tests may be needed if MCH is used as a screening test.

The strength of the present study is that the MCH cut-off level used for differentiating normal from abnormal test was derived from ROC curve, unlike previous reports in which the cut-off level was based on traditional practice. Additionally, unlike previous studies that were conducted on non-pregnant participants, the present study was performed on pregnant women who may have physiologic changes of red blood cells secondary to pregnancy. Therefore, the present results may better represent the effectiveness of the test in real practice because pregnant women are the main target group of screening in prenatal control strategy for severe thalassemia.

The present study also demonstrates MCH is not effective in screening HbE trait because it has low sensitivity (55.3%). Therefore, MCH alone could not effectively detect HbE trait and an additional test such as DCIP (dichlorophenol indophenol) should be performed for such a purpose.

In conclusion, MCH is a good method for screening beta-thalassemia and alpha-thalassemia-1 trait in pregnant women. Because of its high sensitivity and specificity, along with other various aspects such as cost, availability, and technical difficulty, MCH may be one of the available techniques to be used as a primary screening test for thalassemia screening in prevalent areas. However, MCH should be determined by automated hematology analyzer that does not require more personnel or materials. Variation of the result is less than manual method that sometimes depends on personnel and the environment.

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ความไวและความจำเพาะของปริมาณเฉลี่ยของฮีโมโกลบินในเม็ดเลือดแดงในการตรวจคัดกรองพาหะแอลฟาธาลัสซีเมีย-1 และพาหะบีตาธาลัสซีเมีย

สาวิตรี พรานพนัส, สุพัตรา ศิริโชติยะกุล, เกษมศรี ศรีสุพรรณดิฐ, ธีระ ทองสง

วัตถุประสงค์*: เพื่อหาความไว ความจำเพาะ ค่าทำนายผลบวก และค่าทำนายผลลบของปริมาณเฉลี่ยของ ฮีโมโกลบินในเม็ดเลือดแดง (mean corpuscular hemoglobin: MCH) ในการคัดกรองพาหะแอลฟาธาลัสซีเมีย-1 และบีตาธาลัสซีเมีย*

วัสดุและวิธีการ*: เป็นการศึกษาเชิงพรรณนาของการทดสอบในการวินิจฉัย โดยทำการศึกษาในสตรีตั้งครรภ์ 396 รายที่มาฝากครรภ์ในโรงพยาบาลมหาราชนครเชียงใหม่ระหว่างเดือนกันยายน พ.ศ. 2550 ถึง มิถุนายน พ.ศ. 2551 ได้ทำการเก็บเลือดตัวอย่างหลังให้คำปรึกษาและยินยอมเข้าร่วมการศึกษา นำตัวอย่างเลือดไปวิเคราะห์หาระดับ MCH ด้วยเครื่องตรวจโลหิตวิทยาอัตโนมัติ และวัดระดับฮีโมโกลบิน A2 (HbA2) เพื่อวินิจฉัยภาวะบีตาธาลัสซีเมีย และตรวจพีซีอาร์ (PCR) เพื่อตรวจจีนพาหะแอลฟาธาลัสซีเมีย-1 (SEA type) นำข้อมูลมารวบรวม และวิเคราะห์หาค่าความไว ความจำเพาะ ค่าทำนายผลบวก และค่าทำนายผลลบ ในการตรวจคัดกรองดังกล่าว*

ผลการศึกษา*: เมื่อนำข้อมูลมาสร้าง ROC curve พบว่าจุดตัดของ MCH ที่ดีที่สุดในการทำนายพาหะธาลัสซีเมียดังกล่าว คือ 26.5 พิโคกรัม ซึ่งให้ความไว ความจำเพาะ ค่าทำนายผลบวก และค่าทำนายผลลบ ในการตรวจคัดกรองเป็นร้อยละ 95.2, 82.3, 40.4 และ 99.3 ตามลำดับ*

สรุป*: MCH เป็นวิธีที่ดีในการตรวจคัดกรองพาหะแอลฟาธาลัสซีเมีย-1 และบีตาธาลัสซีเมียในสตรีขณะตั้งครรภ์ เนื่องจากมีความไวสูง ทำได้ง่าย และราคาถูก*