Accuracy of Immunochromatographic Strip Test in Diagnosis of Alpha-Thalassemia-1 Carrier

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Objective: *To determine the accuracy of alpha-Thal Immunochromatographic (IC) strip in diagnosis of alpha-thalassemia 1 carrier among pregnant women, using PCR for alpha-thalassemia 1 (SEA type) as a gold standard.*
Material and Method: *Asymptomatic pregnant women attending the antenatal care clinic were recruited. Their blood samples were taken for IC Strip Test (α Thal IC strip, i+Med Laboratories Company Limited) in predicting alpha-thalassemia 1 carrier and separately sent for PCR for diagnosis of α-thalassemia 1 carrier as a gold standard.*
Results: *Four hundred ninety nine pregnant women were recruited into the present study at various gestational weeks. The accuracy of alpha-Thal IC strip test was relatively high as shown in Table 1. Of them, 62 cases were proven to be alpha-thalassemia 1 trait and all of them had the results of positive IC strip, giving a sensitivity of 100%. However, 45 pregnant women of non-α-thalassemia 1 trait had positive test, giving a specificity of 89%.*
Conclusion: *The present study was solid evidence for clinical application of α-thal IC strip in screening program of thalassemia to reduce the need for PCR in diagnosis of α-thalassemia 1 carrier because of its very high sensitivity. The negative test reassures the non α-thalassemia 1 carrier status. Moreover, due to its simplicity, convenience to use, low cost, less-time consuming, clear interpretation and no need for either equipment or expensive laboratories, it may probably be very helpful in a massive screening program.*

Keywords: *Immunochromatographic strip, Thalassemia carrier, Pregnancy*

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Hemoglobin (Hb) Bart's disease (homozygous-α-thalassemia 1) is one of the most common hematologic genetic diseases especially in South East Asia. The prevalence of α-thalassemia 1 gene in the presented population is as high as 10-14%[1,2]. Hb Bart's hydropic fetuses have never survived and their mothers often suffer from obstetric complications such as pre-eclampsia, dystocia, postpartum hemorrhage due to a large placenta, and the psychological burden for carrying a nonviable fetus to term. Each year, our department faces about 20-30 new cases of Hb Bart's disease. Therefore, this disease needs to be controlled, especially by prenatal approach[3]. Recently the authors have had a great success in control of severe thalassemia with a simple way[4] by prenatal diagnosis in risk couples who have been identified by screening strategy.

To achieve the goal of prenatal control strategy, it is necessary to have a highly effective method in identifying thalassemia carriers or a couple at risk. In diagnosis of alpha-thalassemia 1 carriers, various techniques of PCR (polymerase chain reaction) are most commonly used for such a purpose and often used as a gold standard[5]. However, though PCR is associated with reliable accuracy and precision, it has some limitations, preventing them from being

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widely used. For example, PCR is expensive, not widely available especially in the rural areas, need of sophisticated laboratory and experienced or well-trained performers. Therefore, new cheaper techniques to identify α-thal-1 carrier need to be sought after. The authors recently developed a new test, Alpha Thalassemia immunochromatographic strip test (α Thal IC strip, i+Med Laboratories Company Limited) for such a purpose[6]. This is a lateral flow chromatographic immunoassay to determine Hb Bart's in red blood cell hemolysates, which is usually present in a small amount in red blood cells of the carriers of alpha thalassemia 1. This new technique is easy to perform, inexpensive, quick and no equipment necessary and this is the world's first alpha thalassemia immunochromatographic strip test.

Based on pilot test, alpha-Thal IC strip may have sensitivity as high as 100% and specificity of 98%. However, alpha-Thal IC strip test has never been tested in clinical application especially in pregnant women. Because of its simplicity, low cost, no equipment needed, if its accuracy in diagnosis of alpha-thalassemia 1 is comparable to PCR technique, it must be very helpful in a large-scaled screening system. Therefore, this new alpha-Thal IC strip should first be tested for its accuracy together with standard technique to determine its accuracy before being used as a single diagnostic test or screening test for PCR. The objective of the present study was to determine the accuracy of alpha-Thal IC strip in diagnosis of alpha-thalassemia 1 carrier among pregnant women, using PCR for alpha-thalassemia 1 (SEA type) as a gold standard.

Material and Method

The population for the present study was pregnant women attending the antenatal care clinic at first visit at Maharaj Nakorn Chiang Mai Hospital, between October 2007 and May 2008. These pregnant women were routinely screened for thalassemia carriers, as a part of prenatal control of severe thalassemia program[7], which has been established in our hospital for fifteen years. All pregnant women would be invited into the present study. Blood sample of 2 ml were taken and sent separately to different laboratories for the following tests: 1) alpha-Thal IC strip test and 2) DNA analysis for alpha-thalassemia 1 gene (α-thal-1; SEA type) by polymerase chain reaction or PCR technique. The two tests were separately and blindly performed by the two performers at different laboratories.

The definitions used in the present study are as follows: 1) α-thalassemia-1 carrier was an individual, who had deletion of both loci from one chromosome (- -/αα) resulting in clinically in α-thalassemia 1 trait, which is characterized by mimimal hypochromic microcytic anemia or no anemia, usually not associated with clinical abnormality and often goes unrecognized. 2) alpha-Thal IC strip Test: The test Alpha-Thal IC Strip (commercial set; i+LAB aThal IC strip Test) is a qualitative, lateral flow immunoassay for the screening of a minimal amount of Hb Bart's in the specimen, 3) Polymerase chain reaction (PCR) for α-thal-1 (SEA type): PCR in this project used the technique which was modified from Chang's method[5,8] 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 product of 314 and 188 base pair types in blood sample from alpha-thalassemia1 trait.

Screening of α-thalassemia 1 by alpha-thal immuno-chromatographic (IC) strip test

100 µl of EDTA-blood sample and 100 µl of RBC lysis buffer (1% Triton x-100 in distilled water) was added into a 96-well plate and mixed thoroughly. The IC strip was then_immersed vertically in the hemolzyed blood for 2-5 minutes with the arrows on the strip pointing down. After that, the strip was removed and washed until the background was clear using washing buffer (0.05% Tween-PBS) and a wash bottle. The test result was then read visually.

Main outcome measure was accuracy of alpha Thal IC strip in predicting alpha-thalassemia1 carriers, which was calculated and presented as sensitivity, specificity, positive predictive value and negative predictive value.

The present study was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University (Study code: 07SEP051347).

Results

Four hundred ninety nine pregnant women were recruited into the present study at various gestational weeks. The accuracy of alpha-Thal IC strip test was relatively high as shown in Table 1. Of them, 62 cases were alpha-thalassemia1 trait and all of them gave the results of positive IC strip, sensitivity of 100%. However, 45 pregnant women who were proven to be non-α-thalassemia1 trait gave a positive test (false positive rate of 11%). The alpha-Thal IC strip test had specificity of 89.0%.

Table 1. The accuracy of alpha-Thal IC Strip in predicting α-thalassemia 1 carrier among total obstetric population, using PCR as a gold standard

IC-Strip	PCR		Total
	Pos	Neg	
Positive	62	48	110
Negative	0	389	389
Total	62	437	499

Sensitivity: 100% (62/62)
Specificity: 89.0% (389/437)
Positive predictive accuracy: 56.4% (62/110)
Negative predictive accuracy: 100.0% (389/389)
Accuracy 90.0% (451/499) 95% CI: (87.4-92.8%)
Prevalence 12.4% (62/499) 95% CI: (12.4-12.4%)

Discussion

This is the first report on evaluation of the accuracy of IC strip in detection of Hb Bart's in blood samples. It suggests that IC strip has a potential role in clinical application for identifying alpha-thalassemia1 trait that plays an important role in prenatal control of severe thalassemia syndrome. IC strip test is developed to determine a small amount of Hb Bart's qualitatively. Therefore, any conditions or diseases with the presence of Hb Bart's will yield a positive test. Minimal concentration of Hb Bart's in red blood cells is rather specific for alpha-thalassemia 1 trait, though other rare conditions can also be positive. If this test is specific for alpha-thalassemia 1 trait, it will be extremely useful in clinical application to screen or diagnose for an alpha-thalassemia 1 carrier.

In diagnosis of alpha-thalassemia 1 carriers, various techniques of PCR (polymerase chain reaction) are most commonly used for such a purpose and often used as a gold standard[5,7,9-12]. However, though PCR is associated with reliable accuracy and precision, it has some limitations, preventing them from being widely used. For example, PCR technique is expensive, not widely available especially in the rural areas, need of sophisticated laboratory and experienced or well-trained performers. Therefore, a cheaper new test, alpha-Thal IC strip test has been developed for this purpose[6]. However, alpha-Thal IC strip test has never been tested in clinical application especially in pregnant women. Therefore, this new IC strip should first be tested for its accuracy together with standard technique to evaluate its accuracy before clinical application.

The presented results indicated that the IC strip test is very sensitive. All cases with α-thalassemia 1 trait gave a positive result. However, it is not highly specific since several cases other than alpha-thalassemia1 trait could have positive reactivity. Not all positive tests were α-thalassemia1 trait. This is due to the fact that some rare conditions may have a small amount of Hb Bart's in circulation such as Hb H (α-thal 1/α-thal 2), Hb H-CS (α-thal 1/Hb Constant Spring), AE Bart's disease (Hb H disease+HbE trait) and_homozygous alpha-thalassemia 2[6]. Apparently, IC strips could not replace PCR as a diagnostic test since it has some false positive tests. However, it may be used as a screening test for PCR. Due to very high negative predictive value, negative IC strip may obviate the need of PCR. With this approach, PCR could be avoided in more than two-thirds of cases with a positive screening test. The perfect sensitivity and negative predictive value of the IC strip make this test very valuable in identifying cases for further confirmation with PCR. Therefore, the reduction in expense in prenatal control would be substantial.

The PCR that the authors used as gold standard in the present study was specific only for SEA type α-thalassemia1 trait. The authors did not test for other variants of mutations since this is the only type that the authors are concerned. Therefore, it is possible that alpha-thalassemia 1 carrier of other mutation can be missed and this will explain false positive of unknown in some tests. On the other hand, IC strip may also be helpful in other mutations of α-thalassemia 1 since it tests the presence of Hb Bart's in red blood cells regardless mutation types.

The strength of the present study was associated with high reliability of laboratory testing since the technicians performing IC strips and PCR were blinded to each other. Moreover, the sample size was adequate. The result from the present study was solid evidence for clinical application of alpha-thal IC strip in screening program of thalassemia to reduce the need for PCR in diagnosis of α-thalassemia 1 carrier. The negative test reliably excludes alpha-thalassemia 1 carrier status whereas no single case of alpha-thalaseemia 1 carrier will be missed. Moreover, due to its simplicity, convenience to use, low cost, less-time consuming, clear interpretation, and no need for either equipment or expensive laboratories, it may probably be very helpful in a massive screening program.

In conclusion, alpha-thal IC cannot replace PCR. The result from the present study was solid evidence for clinical application of alpha-thal IC strip

in screening program of thalassemia to reduce the need for PCR in diagnosis of alpha-thalassemia 1 carrier especially in the case of a negative test for alpha-thal IC because of its very high negative predictive value. The negative test reassures the non α-thalassemia1 carrier status. Moreover, due to its simplicity, convenience to use, low cost, less-time consuming, clear interpretation, and no need for either equipment or expensive laboratories, it may probably be very helpful in a massive screening program. Alpha-thal IC strip has potential to play an important role to reduce the cost of prenatal control strategy. The authors believe that alpha-thal IC strip can contribute to lower the cost of the prenatal control program.

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Potential conflicts of interest

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ความถูกต้องของการตรวจพาหะของแอลฟาธาลัสซีเมีย-1 ด้วยแถบ alpha-Thal Immunochromatographic (IC)

ชเนนทร์ วนาภิรักษ์, วีรวิทย์ ปิยะมงคล, สุพัตรา ศิริโชติยะกุล, ชัชชัย ตะยาภิวัฒนา, วัชระ กสินฤกษ์, ธีระ ทองสง

วัตถุประสงค์: *เพื่อตรวจสอบความถูกต้องของการวินิจฉัยพาหะของแอลฟาธาลัสซีเมีย-1 ด้วยแถบ alpha-Thal Immunochromatographic (IC) ในหญิงตั้งครรภ์ โดยใช้การตรวจ PCR สำหรับแอลฟาธาลัสซีเมีย-1 (ชนิด SEA) เป็นมาตรฐาน*

วัสดุและวิธีการ: *ได้นำตัวอย่างเลือดของหญิงตั้งครรภ์ที่ไม่มีอาการของโรคธาลัสซีเมียไปตรวจสอบด้วยแถบ IC (alpha-Thal IC strip, i+Med Laboratories Company Limited) เพื่อหาพาหะของ แอลฟาธาลัสซีเมีย-1 และเลือดอีกส่วนหนึ่งได้ส่งเพื่อการตรวจโดย PCR สำหรับการวินิจฉัย แอลฟาธาลัสซีเมีย-1 ซึ่งใช้เป็นมาตรฐาน*

ผลการศึกษา: *ในจำนวนหญิงตั้งครรภ์ 499 ราย ที่เข้าร่วมการศึกษามีอายุครรภ์ต่าง ๆ กันพบว่าความถูกต้องของ alpha-Thal IC strip ในการตรวจสอบมีค่อนข้างสูง ดังแสดงในตารางที่ 1 ในจำนวนนี้มี 62 ราย ที่ได้รับการพิสูจน์ว่าเป็นพาหะของแอลฟาธาลัสซีเมีย-1 ซึ่งพบว่าทุกรายให้ผลบวกกับการตรวจโดยแถบ IC นี้ ส่งผลให้มีความไวร้อยละ 100 อย่างไรก็ตามพบว่ามี 45 ราย ที่ไม่ได้เป็นพาหะของแอลฟาธาลัสซีเมีย-1 แต่ให้ผลบวกกับการตรวจโดยแถบ IC ด้วย ทำให้มีค่าความจำเพาะร้อยละ 89*

สรุป: *การศึกษานี้ทำให้มีหลักฐานที่ชัดเจนสำหรับการประยุกต์ใช้ทางคลินิกของการตรวจหาพาหะแอลฟาธาลัสซีเมีย-1 ด้วยการที่มีความไวสูงมาก ผลลบของการทดสอบจะทำให้มั่นใจได้ว่าไม่ใช่พาหะของแอลฟาธาลัสซีเมีย-1 นอกจากนี้ด้วยความสะดวกต่อการใช้งาน แปลผลง่าย และไม่ต้องการเครื่องมือพิเศษอื่นใดเพิ่มเติมทำให้การตรวจด้วยวิธีนี้น่าจะมีประโยชน์โดยเฉพาะการคัดกรองในกลุ่มประชากรจำนวนมาก*