บทความก่อนของการตรวจกรองพาหะอัลфа-ธาลัสซีเมีย 1 ด้วยแถบชุดทดสอบอิมมูโนโครมาโทกราฟี ในโรงพยาบาลชุมชน

ยุทธ โจนแจ้ง 1 ชายชัย พินเมืองงาม 2 สอน สุชาพงษ์ 3 กัญญาณาภูษณ์ คงไทย 4
บุญตา เข่าวัยรุ่นรัตน์ 5 มัทนี ขวับเสียชีย 6 มิรุติ ศรีวัฒน์นานวงษ์ 2
จินดา พัฒนพงษ์ 7 ปริส แสนบุญ 8 นิรุติ ศิริรัตน์มานะวงศ์ 4
ธวัช ทองน้อย 9 และเพ็ญพักตร์ จันทราภรณ์ 9

บทคัดย่อ
ยีนอัลфа-ธาลัสซีเมีย 1 เป็นยีนชนิดที่มีความรุนแรงของโรคอัลфа-ธาลัสซีเมีย คู่สมรสที่เป็นพาหะอัลфа-ธาลัสซีเมีย 1 จะมีโอกาสทำให้เด็กที่เป็นโรคฮีโมโกลบินบาร์ท ไฮโดรฟิลลิสซึ่งมารดาอาจเกิดภาวะแทรกซ้อนได้หลายอย่าง เช่น ภาวะครรภ์เป็นพิษ และการตกเลือดหลังคลอด ดังนั้นการตรวจวินิจฉัยคัดกรองผู้ที่เป็นพาหะอัลфа-ธาลัสซีเมีย 1 ให้แม่นยำและเร็วจะมีความสัมพันธ์ที่ดีกับการควบคุมการปรากฏของโรคอัลфа-ธาลัสซีเมีย 1 โดยการตรวจโปรตีนฮีโมโกลบินบาร์ทในเลือด เนื่องจากมีความสำคัญ อย่างไรก็ตาม ยังไม่มีรายงานเกี่ยวกับการศึกษาความถูกต้องของ αTHAL IC strip (αTHAL IC strip, i+Med Laboratories Company Limited) เป็นชุดทดสอบอิมมูโนโครมาโทกราฟีที่มีความถูกต้อง 100% โดยการตรวจพาหะอัลфа-ธาลัสซีเมีย 1 ได้แสดงให้เห็นว่าชุดทดสอบอิมมูโนโครมาโทกราฟี เช่น αTHAL IC strip สามารถตรวจพาหะอัลфа-ธาลัสซีเมีย 1 ได้อย่างแม่นยำและรวดเร็ว โดยไม่ต้องใช้เทคโนโลยีใด ๆ เพิ่มเติมในการตรวจ สามารถใช้ได้ทุกที่ และไม่ต้องใช้การเตรียมตัวอย่างใด ๆ ที่ซับซ้อน

คำาสำาคัญ:
ชุดทดสอบอัลфа-ธาลัสซีเมีย อิมมูโนโครมาโทกราฟี พาหะอัลфа-ธาลัสซีเมีย 1 ความถูกต้อง โรงพยาบาลชุมชน

*ผู้รับผิดชอบบทความ E-mail address: drjohpc9@gmail.com
ธาลัสซีเมียผิดปกติ จำนวน 2,027 ราย ต่อไปตรวจสอบพาหะอัลфа-ธาลัสซีเมีย 1 ด้วย αTHAL IC strip และตรวจยืนยันโดยวิธีพีซีอาร์ที่ศูนย์อนามัยเขต กรมอนามัย พบว่าแนวทางที่ 1 การตรวจกรองพาหะอัลфа-ธาลัสซีเมีย 1 ด้วย αTHAL IC strip มีค่าความไวอยู่ในช่วงร้อยละ 83.3-100 ค่าความล่าเฉลยอยู่ในช่วงร้อยละ 72.0-89.9 และค่าความถูกต้องอยู่ในช่วงร้อยละ 73.2-89.7 และแนวทางที่ 2 มีค่าความไวอยู่ในช่วงร้อยละ 76.2-100 ค่าความล่าเฉลยอยู่ในช่วงร้อยละ 68.3-87.2 และค่าความถูกต้องอยู่ในช่วงร้อยละ 72.2-88.7 ซึ่งจะเห็นได้ว่าแนวทางที่ 2 มีอัตราการเกิดผลลบปลอมสูงกว่าแนวทางที่ 1 การศึกษาในครั้งนี้สรุปได้ว่า αTHAL IC strip มีประสิทธิภาพในการใช้ตรวจกรองพาหะอัลфа-ธาลัสซีเมีย 1 ในโรงพยาบาลชุมชน เนื่องจากมีความถูกต้องสูง แต่ต้องใช้ตัวอย่างเลือดที่เป็นเลือดใหม่ นอกจากนี้การทำเพิ่ม αTHAL IC strip ตามหลังการตรวจกรองปกติด้วย MCV/DCIP จะสามารถลดจำนวนตัวอย่างที่จะส่งตรวจวิเคราะห์ดีเอ็นเอได้ ส่วนต้นทุน-ประสิทธิผลควรจะมีการศึกษาอย่างละเอียดต่อไป
Accuracy of Immunochromatographic Strip Test for Screening of $\alpha$-thalassemia 1 Carriers at the Rural Clinical Settings

Yupin Jopang$^1*$, Chanchai Pinmuang-ngam$^2$, Sanong Suksaweang$^3$, Kunyakan Khongthai$^4$, Busaba Techachainiran$^5$, Mattanee Chewasateanchai$^6$, Nirut Siriratmanawong$^2$, Jintana Pattanapongthorn$^7$, Paripat Netnee$^1$, Vanida Kamonsin$^1$, Thawat Thongnoi$^8$, Arak Deemai$^9$, Varangnuch Jitpakdeebodin$^{10}$, Jintana Uedjantuk$^{11}$ and Penpak Chantaraporn$^{12}$

$^1$Regional Health Promotion Center 9, Nakhon Ratchasima, $^2$Regional Health Promotion Center 3, Nakhon Sawan, $^3$Department of Pathology and Laboratory Medicine, Institute of Medicine, Suranaree University of Technology, $^4$Regional Health Promotion Center 1, Chiang Mai, $^5$Regional Health Promotion Center 5, Ratchaburi, $^6$Regional Health Promotion Center 7, Khon Kaen, $^7$Bureau of Health Promotion, Department of Health, $^8$Pakchong Nana Hospital, $^9$Dankhuntod Hospital, $^{10}$Maharat Hospital, $^{11}$Sikhiu Hospital, $^{12}$Sungnoen Hospital, Nakhon Ratchasima Province

Abstract

$\alpha$-thalassemia 1 gene is the severe form of $\alpha$-thalassemia. Couples with carrier of this gene are at risk of having hemoglobin Bart’s hydrops fetalis and the mother often suffers from complications such as pre-eclampsia and postpartum hemorrhage. Therefore, the screening of $\alpha$-thalassemia 1 carrier in pregnant women and their partners is very important. The $\alpha$-thalassemia immunochromatographic strip test (\$\alpha$THAL IC strip, i+Med Laboratories Company Limited) might be an alternative tool to identify carriers of $\alpha$-thalassemia 1 by testing for hemoglobin Bart’s because it is convenient, cheap and less-time consuming. However, the accuracy of this screening method has not been tested in a large-scale at the rural clinical settings as multi-centers. This study aimed to determine the accuracy of $\alpha$THAL IC strip for screening $\alpha$-thalassemia 1 in two protocols. Protocol 1, a total of 898 fresh blood samples of pregnant

Keywords: $\alpha$-thalassemia immunochromatographic strip test, $\alpha$-thalassemia 1 carrier, accuracy, rural clinical settings

*Corresponding author E-mail address: drjohpe9@gmail.com
women and their partners were screened. They were sent to our laboratory for further confirmation by polymerase chain reaction (PCR) technique. Protocol II, a total of 2,027 aged samples with positive results by the screening tests during the national prevention and control program. They were further tested for α-thalassemia 1 carrier with the αTHAL IC strip and DNA analysis at the Regional Health Promotion Centers, Department of Health. The sensitivity, specificity and accuracy of the αTHAL IC strip for screening α-thalassemia 1 carriers by protocol I were 83.3-100%, 72.0-89.9% and 73.2-89.7% respectively, whereas protocol II were 76.2-100%, 68.3-87.2% and 72.2-88.7%, respectively. It was found that the screening by protocol II had higher false negative results than by protocol I. Taken together; the study revealed that the αTHAL IC strip could be used effectively for screening α-thalassemia 1 carriers due to its high accuracy at the rural clinical settings with fresh blood samples. In addition, the protocol with application of the IC strip test after regular MCV/DCIP screening existing could as well help reduce the number of further DNA analysis samples. However, the cost-effectiveness should be further evaluated extensively.
Introduction

In α-thalassemia diseases, α-thalassemia 1 gene is the most severe form, and it represents a major public health problem in many areas of the world, including Southeast Asia and China. In Thailand, the prevalence of α-thalassemia 1 gene varies from 3.6 to 10% in different regions of the country.\(^{(1)}\) A couple who carry this gene are at risk of having a fetus with severe anemia in utero, leading to death in late gestation or shortly after birth, namely hemoglobin Bart’s hydrops fetalis. Moreover, their affected mothers often suffer from complications such as pre-eclampsia and postpartum hemorrhage.\(^{(2)}\) Therefore, a counseling with the identification of α-thalassemia 1 gene (or gene product) before having a child is an important step to prevent and control the disease. To identify such gene, it would require a highly effective method such as the practically widely used polymerase chain reaction (PCR).\(^{(3-5)}\) But, this method can be problematic in rural areas where the PCR technique is not always available due to the high cost per test, the need of sophisticated laboratory equipment and the need of experienced or well-trained medical technologists.

Several studies have well demonstrated that thalassemia immunochromatographic strip test (αTHAL IC strip, i+Med Laboratories Company Limited) might be an alternative and attractive tool to identify carriers of α-thalassemia 1. The strip was purposely applied to test for Hb Bart’s (γ₄) in blood samples at any setting due to it being convenient, inexpensive and less time-consuming.\(^{(6-8)}\) However, none of the previous studies has been tested in a large-scale at the practical multi-rural clinical settings. The objective of this study was to determine the accuracy of αTHAL IC strip for screening α-thalassemia 1 carrier among pregnant women and their partners from 5 different laboratories of community hospitals and 5 laboratories of the Regional Health Promotion Centers (HPC), Department of Health, Ministry of Public Health, Thailand, by using PCR for α-thalassemia 1 (SEA and THAI type) as a gold standard.

Materials and Methods

The proposal had been approved by Clinical Research Ethical Committee, HPC 3, Nakhon Sawan, Thailand (EC 011/2013) before the starting of this study.

The study was conducted in two different protocols based on the duration the blood samples were obtained. Protocol I: a total of 898 fresh EDTA-anticoagulated blood samples of pregnant women and their partners collected at antenatal care clinic during September to October 2014 at 5 community hospitals were screened α-thalassemia 1 carriers with the αTHAL IC strip by medical technologists within 24 hours after the blood samples were drawn. Then, all the screened
samples were transferred to our laboratory at the HPC 9, Nakhon Ratchasima for further confirmation with real-time gap-PCR with SYBR Green I and high resolution melting (HRM) analysis. Protocol II: a total of 2,027 aged EDTA-anticoagulated blood samples with positive initial screening via automated blood cell counters and DCIP-tests as previously described during the prevention and control program were sent from community hospitals to 5 laboratories of the HPC, Department of Health, established in many regions of the country between January to August, 2014. All samples were sent by various means of transportation before they were tested α-thalassemia 1 carriers with the αTHAL IC strip and by PCR technique. Note that the two tests of each sample were separately and blindly performed by two different medical technologists.

Screening of α-thalassemia 1 by αTHAL immunochromatographic (IC) strip test

We used the iLAB αTHAL IC strip test kit by applying the IC strips, reagents, and a protocol provided by the manufacturer (i+Med Laboratories Company Limited, Bangkok, Thailand). In brief, 200 μl of EDTA-blood sample was mixed with lysis solution containing 1% Triton X-100 to break all the red blood cells. Then, the IC strip was immersed vertically in the hemolysate for about 2-5 min. Next, the IC strip was removed from the blood sample and washed with washing buffer until the background was clear as recommended in the leaflet. The result was finally read by naked eyes. A positive result would have two pink bands appear at the control and the test zones whereas a negative sample would appear a pink band only at the control zone (Fig. 1).

Fig. 1 A representative IC strip assay for detection of Hb Bart’s. P and N are positive and negative control results, respectively. 1 and 3 are very weakly positive results, 2 and 5 are negative results and 4 is positive result with α-thalassemia 1 carrier.
Results

The protocol I, out of total 898 subjects investigated by the 5 different laboratories at community hospitals, 28 cases were identified as α-thalassemia 1 carriers. One of them gave a negative result by IC strip but positive by PCR. The sensitivity, specificity and accuracy of the αTHAL IC strip for screening α-thalassemia 1 carriers were 83.3-100%, 72.0-89.9% and 73.2-89.7%, respectively (Table 1).

On the other hand, from 2,027 subjects recruited into the protocol II, 326 cases were identified as α-thalassemia 1 carriers but 19 cases gave negative results by IC strip while tested positive with PCR. The sensitivity, specificity and accuracy of the αThaL IC strip for screening α-thalassemia 1 carriers were 76.2-100%, 68.3-87.2% and 72.2-88.7%, respectively (Table 2).

Table 1 The accuracy of αTHAL IC strip assay for Hb Bart’s in the detection of α–thalassemia 1 carriers with protocol I among 5 different community hospitals, using real-time gap-PCR with SYBR Green I and HRM analysis for α–thalassemia 1 (SEA & THAI deletion) as a gold standard

<table>
<thead>
<tr>
<th>IC strip assay</th>
<th>Hospital A</th>
<th>Hospital B</th>
<th>Hospital C</th>
<th>Hospital D</th>
<th>Hospital E</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR analysis</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>49</td>
<td>38</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>(8/8) x 100 = 100%</td>
<td>(4/4) x 100 = 100%</td>
<td>(3/3) x 100 = 100%</td>
<td>(7/7) x 100 = 100%</td>
<td>(5/6) x 100 = 83.3%</td>
</tr>
<tr>
<td>Specificity</td>
<td>(126/175)x100= 72.0%</td>
<td>(139/173)x100= 80.3%</td>
<td>(124/153)x100= 81.0%</td>
<td>(141/171)x100= 82.4%</td>
<td>(178/198)x100= 89.9%</td>
</tr>
<tr>
<td>PPV</td>
<td>(8/57)x100= 14.0%</td>
<td>(4/38)x100= 10.5%</td>
<td>(3/32)x100= 9.4%</td>
<td>(7/37)x100= 18.9%</td>
<td>(5/25)x100= 20.0%</td>
</tr>
<tr>
<td>NPV</td>
<td>(126/128)x100=100%</td>
<td>(139/139)x100=100%</td>
<td>(124/124)x100=100%</td>
<td>(141/141)x100=100%</td>
<td>(178/178)x100=100%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>(134/183)x100=73.2%</td>
<td>(143/177)x100=80.8%</td>
<td>(127/156)x100=81.4%</td>
<td>(148/178)x100=83.1%</td>
<td>(183/204)x100=89.7%</td>
</tr>
</tbody>
</table>

Note: +, positive; -, negative; PPV, positive predictive value; NPV, negative predictive value
Discussion

This is the first report on evaluation of the accuracy of IC strip in the detection of α-thalassemia 1 carrier with protocol II among the different 5 Regional Health Promotion Centers, Department of Health, using PCR analysis for α-thalassemia 1 (SEA & THAI deletion) as a gold standard. The results revealed that an accuracy of IC strip test will be better in case of using fresh blood specimens collected within 24 hours (Protocol I) than those aged blood specimens (Protocol II). Only one of 898 (0.11%) fresh samples gave false negative result whereas much higher number was found in aged samples, 19 of 2027, (0.94%). We, therefore, would like to emphasize that one should never use aged blood to test with the IC strip rather than fresh collected blood as recommended by the manufacturer. The optimal place to perform the IC strip test would be best at the rural community hospitals where the blood samples are freshly taken. However, the false negative result possibly still occurred with the IC strip test that was read by naked eyes. In fact, a false negative result like this is not preferable during any screening test. It could probably happen that the reader could not see the very weak band well enough if the background was not well-cleared. We suggested that the visual detection of such positive result must be trained properly.

In contrast, the results of the present study from protocol I showed that the IC strip test had high proportions of samples with non-alpha-thalassemia 1 (SEA and Thai type) deletions but positive for IC strip test, 162 of 898 samples (18%). The test itself was designed to detect Hb Bart’s at a concentration of α-thalassemia 1 carrier with protocol II among the different 5 Regional Health Promotion Centers, Department of Health, using PCR analysis for α-thalassemia 1 (SEA & THAI deletion) as a gold standard.
> 5 μg/ml\(^{(1)}\), therefore, the other thalassemia diseases that contain Hb Bart’s in blood circulation such as Hb H (α-thal 1/ α-thal 2), Hb H-CS (α-thal 1/Hb Constant spring), AEBart’s disease, homozygous α-thalassemia 2 and some heterozygous α-thalassemia 2 would give a positive result. Unfortunately, this seems to be inappropriate to use in the high prevalence area of α-thalassemia 2 gene, especially the northeast of Thailand where the frequency of α-thalassemia 2 gene is high.\(^{(12)}\)

The results are similar to previous studies even with a larger sample size in terms of sensitivity, specificity and accuracy respectively.\(^{(6-8)}\) However, we did not differentiate the phenotypes of those samples with non-alpha-thalassemia 1 (SEA and Thai type) deletions but positive for IC strip test samples because of the limitation of budget.

In Thailand, practically, the three severe thalassemia diseases; homozygous α-thalassemia (Hb Bart’s hydrops fetalis), homozygous β-thalassemia, and β-thalassemia/ Hb E disease are the prime targets for the prevention and control strategy. A screening protocol is done based on blood indices from an electronic blood cell counting and dichlorophenolindophenol (DCIP) precipitation test. Individuals with low MCV (<80 fL) or a positive DCIP test are initially considered as positive screenings and will be further investigated with Hb analysis and DNA analysis. It is known that MCV<80 fL is effectively used for screening α-thalassemia 1 and β-thalassemia whereas DCIP test is used for screening Hb E. In addition, 25-35% Hb E level has been proved no coexistence of α-thalassemia 1 in the same individual, no need for DNA analysis.\(^{(9)}\) It is noteworthy, as shown in Fig. 2, that applying the IC strip assay to screen α-thalassemia 1 carriers after a regular MCV / DCIP screening existing protocol, only 96 of 329 samples had positive results and need further DNA analysis. In comparison, without the IC strip test, 180 of 329 samples gave positive results by a conventional screening protocol using MCV and DCIP tests and already excluded the number of samples with 25-35% Hb E level before further confirming by DNA analysis. According to the recommendation by Winichagoon P, et al. study.\(^{(8)}\) if the types of Hb are HbH, HbH-CS, EABart’s, EFBart’s, CSEABart’s and CSEFBart’s, DNA analysis for α-thalassemia 1 will not need to be continued. However, due to the limitation of resource in real situation at rural clinical setting that Hb analysis will be done mostly at provincial hospitals or thalassemia centers, such suggestion may not be applicable. Our results also showed that the number of positive screening samples for further DNA analysis reduced almost half with the IC strip adding. Therefore, it is fair to conclude that combine the IC strip assay with an existing MCV / DCIP protocol could rule out mass populations for further α-thalassemia 1 detection by PCR analysis. In terms of cost-effectiveness in this study, the estimated total cost of the protocol
with IC strip test was 73,080 baht [(329 cases x 120 baht) + (96 cases x 350 baht)] compared to the protocol without IC strip test of 63,000 baht (180 cases x 350 baht). It seems that the protocol with IC strip test is more expensive than the protocol without IC strip test, but this has not included the cost of the machines and labor cost.

**Conclusion**

Taken together, we would like to conclude that the iLAB αTHAL IC Strip Test showed a high accuracy (>70%) for screening α-thalassemia 1 carriers in practical multi-rural clinical settings. In addition, we would recommend that aged samples are not proper for the iLAB αTHAL IC Strip Test for screening α-thalassemia 1 because false negative results may occur, which is not preferred during a screening process. To verify a complete cost-effectiveness, extensive studies on all relevant factors should be further evaluated in the future.

**Acknowledgements**

This work was supported by the National Health Security Office, Thailand. We would like to thank the i+Med Laboratories Company Limited, Bangkok, Thailand for providing the IC strip assay kits used in this study.
References


