วิจารณ์และสรุปผลการศึกษา

ในการศึกษาครั้งนี้ได้แบ่งผลการศึกษาออกเป็น 2 ส่วน คือ ส่วนที่ 1 ผลการศึกษาจากการเก็บตัวอย่างเลือดครั้งที่ 1 ซึ่งทำให้ทราบข้อมูลพื้นฐานของธาลัสซีเมีย ภาวะเลือดจาง และภาวะขาดเหล็กในกลุ่มหญิงตั้งครรภ์ที่ศึกษา และส่วนที่ 2 ผลจากการติดตามเก็บตัวอย่างเลือดครั้งที่ 2 ซึ่งเป็นผลการศึกษาเพื่อประเมินผลของยีนบีตาอีโกลบินที่มีต่อการตอบสนองของพารามิเตอร์ทางโลหิตวิทยาจากการได้รับเหล็กเสริมในระหว่างการตั้งครรภ์

จากการเก็บตัวอย่างเลือดหญิงตั้งครรภ์ทุกรายที่เข้าเกณฑ์ (inclusion criteria) ที่มาฝากครรภ์ที่โรงพยาบาลอำเภอชุมแพ และโรงพยาบาลอำเภอน้ำพองในระหว่างเดือนมกราคม 2546 ถึง เดือนพฤษภาคม 2547 ได้ตัวอย่างเลือดทั้งหมด 481 ราย ผลการศึกษาพบหญิงตั้งครรภ์ที่มียีนธาลัสซีเมียสูงถึงร้อยละ 61.7 และชนิดของยีนธาลัสซีเมียที่พบสูงที่สุดก็คือ β^{E}-blobin gene ตามด้วย α^{+}-thalassemia, α^{CS}, α^{0}-thalassemia, $\alpha^{Paksé}$ และ β-thalassemia รวมทั้งพบยีนฮีโมโกลบินผิดปกติซึ่งพบได้ไม่บ่อยนัก คือ $\alpha^{Q\text{-}Thailand}$ ซึ่งเมื่อยีนเหล่านี้มีปฏิสัมพันธ์กันทำให้ตรวจพบจีโนไทป์ที่แตกต่างกันถึง 24 ชนิด (ตารางที่ 1) ซึ่งสอดคล้องกับผลการศึกษาของ Fucharoen G และคณะฯ (19) ที่ได้ทำการศึกษายีนธาลัสซีเมียในกลุ่มตัวอย่างเลือดชาวไทยเชื้อสายเขมร ในจังหวัดสุรินทร์และบุรีรัมย์ ที่พบจีโนไทป์ของธาลัสซีเมียที่แตกต่างกันถึง 22 ชนิด แสดงให้เห็นถึงความหลากหลายของธาลัสซีเมียในกลุ่มประชากรภาคอิสานได้เป็นอย่างดี และสะท้อนให้เห็นถึงความจำเป็นที่จะต้องมีวิธีการตรวจกรองธาลัสซีเมียที่เหมาะสมที่จะใช้ในการดำเนินการควบคุมและป้องกันโรคเลือดจางธาลัสซีเมียชนิดรุนแรงในพื้นที่ ในการศึกษาครั้งนี้จึงได้ทำการประเมินผลการตรวจกรองธาลัสซีเมียในกลุ่มหญิงตั้งครรภ์ที่ศึกษาด้วยชุดน้ำยาสำเร็จรูป KKU-OF และ KKU-DCIP-Clear เปรียบเทียบกับแนวทางการตรวจกรองโดยใช้ค่าดัชนีเม็ดเลือดแดงที่แตกต่างกัน 4 แนวทาง พบว่าการตรวจกรองด้วยชุดน้ำยาสำเร็จรูป KKU-OF และ KKU-DCIP-Clear เป็นแนวทางที่ให้ผลความไวและความจำเพาะสูงที่สุด (ดูรายละเอียดของผลการศึกษาในภาคผนวก) เป็นข้อมูลที่ยืนยันอย่างชัดเจนว่าแนวทางการตรวจกรองด้วยชุดน้ำยาสำเร็จรูป KKU-OF และ KKU-DCIP-Clear ที่ใช้อยู่ในแผนงานควบคุมและป้องกันโรคเลือดจางธาลัสซีเมียเป็นแนวทางที่เหมาะสมสำหรับพื้นที่ที่มีความหลากหลายของธาลัสซีเมียและมีข้อจำกัดทางด้านบุคลากรและงบประมาณดังเช่นในประเทศไทยและประเทศในแถบเอเชียอาคเนย์ที่มีการกระจายของธาลัสซีเมียในลักษณะเดียวกัน

จากการเปรียบเทียบข้อมูลทางโลหิตวิทยาในกลุ่มหญิงตั้งครรภ์ที่มีธาลัสซีเมียจีโนไทป์ต่าง ๆ ในการศึกษานี้ กับข้อมูลทางโลหิตวิทยาในกลุ่มตัวอย่างที่มีจีโนไทป์ชนิดเดียวกันที่ไม่ใช่หญิงตั้งครรภ์ (19) พบว่าไม่แตกต่างกัน บ่งชี้ว่าภาวะการตั้งครรภ์ที่มีอายุครรภ์น้อยกว่า 20 สัปดาห์นั้น ไม่น่าจะมีผลต่อข้อมูลทางโลหิตวิทยาแต่อย่างใด ดังนั้น หากต้องการตรวจกรองธาลัสซีเมียในหญิงตั้งครรภ์ที่มีอายุครรภ์น้อยกว่า 20 สัปดาห์โดยใช้ค่าดัชนีเม็ดเลือดแดง จึงสามารถใช้ค่า cutoff เดียวกันกับที่ใช้อยู่ในประชากรทั่วไปได้ ซึ่งจากผลการศึกษาเปรียบเทียบแนวทางการตรวจกรองธาลัสซีเมียข้างต้นพบว่าการใช้ค่า MCV < 80 fl หรือ MCH < 27 pg (28, 29) สามารถคัดกรองเอาหญิงตั้งครรภ์ที่เป็น

พาหะอัลฟาธาลัสซีเมีย 1 และบีตาธาลัสซีเมียเข้ามาได้ทั้งหมด แต่จะมีพาหะของฮีโมโกลบินอีบางส่วนที่ไม่ถูกคัดกรองเข้ามา ดังนั้นจึงจำเป็นที่ต้องใช้การทดสอบอื่นในการตรวจหาพาหะของฮีโมโกลบินอี ซึ่งพบว่าการใช้ค่าดัชนีเม็ดเลือดแดงร่วมกับชุดน้ำยาสำเร็จรูป KKU-DCIP-Clear ก็เป็นอีกแนวทางหนึ่งที่ให้ผลดีเช่นเดียวกัน

เมื่อทำการประเมินภาวะเลือดจางในกลุ่มหญิงตั้งครรภ์ที่ศึกษา โดยใช้เกณฑ์ Hb < 11 g/dl พบความชุกของภาวะเลือดจางร้อยละ 23.8 ซึ่งสอดคล้องกับรายงานภาวะเลือดจางในหญิงตั้งครรภ์ที่มาฝากครรภ์ที่โรงพยาบาลศรีนครินทร์ มหาวิทยาลัยขอนแก่น (30) และเมื่อพิจารณาผลการตรวจพบยีนธาลัสซีเมียในกลุ่มหญิงตั้งครรภ์ที่ศึกษาทั้งที่มีและไม่มีภาวะเลือดจาง จะเห็นว่าสัดส่วนของหญิงตั้งครรภ์ที่มียีนธาลัสซีเมียในกลุ่มที่มีภาวะเลือดจางสูงกว่าในกลุ่มที่ไม่มีภาวะเลือดจางอย่างชัดเจน (ร้อยละ 83.7 vs ร้อยละ 53.5) เมื่อพิจารณาสัดส่วนของผู้ที่มีภาวะขาดเหล็กและผู้ที่มียีนธาลัสซีเมียในกลุ่มหญิงตั้งครรภ์ที่มีภาวะเลือดจางพบว่า ร้อยละ 31.6 (31/98) มีภาวะขาดเหล็ก (แบ่งเป็นผู้ที่มีภาวะขาดเหล็กเพียงอย่างเดียวร้อยละ 9.2 และมีภาวะขาดเหล็กร่วมกับยีนธาลัสซีเมียร้อยละ 22.4) และร้อยละ 61.2 (60/98) มียีนธาลัสซีเมีย ส่วนที่เหลืออีกร้อยละ 7.1 (7/98) ที่ไม่พบทั้งยีนธาลัสซีเมียและภาวะขาดเหล็กน่าจะมีภาวะเลือดจางจากสาเหตุอื่น ข้อมูลจากการศึกษาครั้งนี้บ่งชี้ว่าภาวะเลือดจางในหญิงตั้งครรภ์ที่ตรวจพบในกลุ่มประชากรนี้น่าจะเกิดจากยีนธาลัสซีเมียมากกว่าภาวะขาดเหล็ก และน่าจะเป็นสาเหตุหนึ่งที่ทำให้ยังตรวจพบความชุกของภาวะเลือดจางได้ค่อนข้างสูงในกลุ่มประชากรนี้

จากการวิเคราะห์ข้อมูลด้วยสถิติการถดถอยโลจิสติก โดยมีการควบคุมผลจากภาวะขาดเหล็ก อายุ อายุครรภ์ และดัชนีมวลกาย เพื่อประเมินผลของยีนธาลัสซีเมียชนิดต่าง ๆที่มีต่อภาวะเลือดจาง จะเห็นว่านอกเหนือจากกลุ่มที่มีมียีนผิดปกติชนิดเดียวกันมากกว่าหรือเท่ากับ 2 ยีน (heterozygous α^0-thalassemia, compound heterozygous $\alpha^0/\alpha^{CS/Ps}$, compound heterozygous α^0/α^+ และ homozygous Hb E) ซึ่งเป็นที่ทราบกันดีอยู่แล้วว่าจะส่งผลให้เกิดภาวะเลือดจางหรือโรคธาลัสซีเมียได้ (1) ยังพบว่าพาหะฮีโมโกลบินอีและพาหะฮีโมโกลบินคอนสแตนท์สปริงหรือพาหะฮีโมโกลบินปากเซก็มีผลต่อการเกิดภาวะเลือดจางอย่างมีนัยสำคัญ ในขณะที่พาหะของอัลฟาธาลัสซีเมีย 2 (heterozygous α^+-thalassemia), พาหะฮีโมโกลบินอีร่วมกับพาหะอัลฟาธาลัสซีเมีย 2 และพาหะฮีโมโกลบินอีร่วมกับพาหะฮีโมโกลบินคอนสแตนท์สปริงหรือฮีโมโกลบินปากเซนั้นไม่มีผลต่อการเกิดภาวะเลือดจาง จากข้อมูลที่ได้นี้สะท้อนให้เห็นว่าทั้งฮีโมโกลบินอีและฮีโมโกลบินคอนสแตนท์สปริงถึงแม้จะเป็นเพียงแค่พาหะก็เป็นสาเหตุการเกิดภาวะเลือดจางในหญิงตั้งครรภ์ด้วย อย่างไรก็ตามการมีปฏิสัมพันธ์ของฮีโมโกลบินอีร่วมกับการมียีนอัลฟาธาลัสซีเมียที่ผิดปกติไปเพียงหนึ่งยีน (คือร่วมกับยีน α^+ หรือร่วมกับยีน $\alpha^{CS/Ps}$) กลับเป็นผลดีที่ทำให้เกิดภาวะเลือดจางได้น้อยลง ทั้งนี้น่าจะเป็นผลสืบเนื่องมาจากการที่มีการสร้างสายโกลบินลดลงทั้งสายอัลฟาและสายบีตา จึงเป็นผลให้มีการจับกันระหว่างสายโกลบินทั้ง 2 ชนิดมีความสมดุล ไม่มีสายใดสายหนึ่งเหลือมากเกินไปที่จะก่อให้เกิดพยาธิสภาพต่อเม็ดเลือดแดงและทำให้เม็ดเลือดแดงแตกได้ (1)

จากการเปรียบเทียบค่าความต่างระหว่าง 2 กลุ่มอย่างหยาบ โดยที่ยังไม่ได้ควบคุมผลกระทบจากปัจจัยอื่นๆ พบว่า มีเพียงค่าการเปลี่นแปลงของค่า MCV (dMCV) เท่านั้น ที่มีความแตกต่างระหว่าง 2 กลุ่มอย่างมีนัยสำคัญ โดยที่จะพบว่า ค่า MCV ในหญิงตั้งครรภ์ที่ไม่มียีนธาลัสซีเมียเพิ่มขึ้นมากกว่าค่า MCV ของหญิงตั้งครรภ์ที่มียีนฮีโมโกลบินอี ซึ่งเป็นไปในลักษณะเดียวกันกับการเปลี่ยนแปลงของค่า MCV ในหญิงตั้งครรภ์ที่เป็นพาหะบีตาธาลัสซีเมียที่เคยมีรายงานมาก่อน (31) น่าจะเป็นข้อบ่งชี้ว่าความสามารถในการสร้างฮีโมโกลบินภายในเซลล์เม็ดเลือดแดงในผู้ที่มียีนฮีโมโกลบินอีด้อยกว่าผู้ที่ไม่มียีนธาลัสซีเมียถึงแม้จะได้รับเหล็กเสริมเช่นเดียวกัน แสดงให้เห็นถึงมีการตอบสนองต่อการได้รับเหล็กเสริมที่น้อยกว่า และเมื่อทำการวิเคราะห์ตัวแปรเชิงพหุ โดยมีการควบคุมผลกระทบจากอายุ อายุครรภ์ ดัชนีมวลกาย ระดับเฟอร์ไรทิน และระยะเวลาที่ได้รับเหล็กเสริม พบว่านอกเหนือจากการเปลี่ยนแปลงของค่า MCV (dMCV) แล้ว ค่าการเปลี่ยนแปลงของค่า Hb, MCH และค่า RDW (dHb, dMCH, dRDW) ของกลุ่มหญิงตั้งครรภ์ที่มียีนบีตาอีมีการตอบสนองต่อการได้รับเหล็กเสริมน้อยกว่าหญิงตั้งครรภ์ที่ไม่มียีนธาลัสซีเมียอย่างมีนัยสำคัญ (ตารางที่ 9) อย่างไรก็ตาม เนื่องจากในกลุ่มหญิงตั้งครรภ์ที่มียีนบีตาอีในการศึกษาครั้งนี้ประกอบไปด้วยจีโนไทป์ที่แตกต่างกันหลายแบบ จึงได้ทำการวิเคราะห์ข้อมูลซ้ำอีกครั้ง เพื่อประเมินผลของการมียีนบีตาอีโกลบินที่มีจีโนไทป์ที่แตกต่างกัน โดยแบ่งกลุ่มหญิงตั้งครรภ์ที่มียีนบีตาอีโกลบินออกเป็นกลุ่มย่อยอีก 3 กลุ่ม คือ พาหะฮีโมโกลบินอี (heterozygous Hb E), พาหะฮีโมโกลบินอีที่มียีนอัลฟาธาลัสซีเมียร่วม (heterozygous Hb E co-inherited with α-thalassemia) และโฮโมไซกัสฮีโมโกลบินอี (homozygous Hb E) พบว่า ในหญิงตั้งครรภ์ที่มียีนบีตาอีทุกแบบ จะมีการตอบสนองของค่า MCV และ MCH ที่แตกต่างจากหญิงตั้งครรภ์ปกติอย่างมีนัยสำคัญทางสถิติ ส่วนการตอบสนองของค่า Hb นั้น พบว่า เฉพาะกลุ่มหญิงตั้งครรภ์ที่มียีนบีตาอีโกลบินแบบโฮโมไซโกทเท่านั้น ที่แตกต่างจากกลุ่มปกติอย่างมีนัยสำคัญ (ตารางที่ 10) ซึ่งเป็นข้อมูลที่ยืนยันอีกครั้งว่าหญิงตั้งครรภ์ที่มียีนบีตาอีมีความสามารถในการสร้างฮีโมโกลบินภายในเซลล์ได้น้อยกว่าหญิงตั้งครรภ์ปกติ ถึงแม้ว่าจะได้รับเหล็กเสริมเช่นเดียวกับหญิงตั้งครรภ์ปกติก็ตาม ทั้งนี้น่าจะเป็นไปตามสมมติฐานที่ว่าการสร้างฮีโมโกลบินภายในเซลล์เม็ดเลือดแดงในผู้ที่มียีนธาลัสซีเมียจะสร้างได้น้อยกว่าคนปกติ เนื่องจากมีการสร้างสายโกลบินสายใดสายหนึ่งลดลง ทำให้มีสายโกลบินที่จะไปจับกันเป็นโมเลกุลของฮีโมโกลบินลดน้อยลงไปด้วย (1) ซึ่งจะส่งผลให้มีค่า MCV และ MCH ต่ำกว่าผู้ที่ไม่มียีนธาลัสซีเมียอย่างชัดเจน (ตารางที่ 4) ดังนั้น หากหญิงตั้งครรภ์เหล่านี้ไม่มีภาวะขาดเหล็กร่วมด้วยแล้ว ถึงแม้จะได้รับเหล็กเสริมเข้าไปก็ไม่ส่งผลให้มีการสร้างฮีโมโกลบินได้ภายในเซลล์เพิ่มมากขึ้น และจะส่งผลต่อค่าฮีโมโกลบินรวม (Hb) ในกลุ่มที่เป็นโฮโมไซกัสฮีโมโกลบินอี แสดงให้เห็นว่าหญิงตั้งครรภ์ที่มียีนบีตาอีโกลบิน โดยเฉพาะอย่างยิ่งกรณีที่มียีนบีตาอีโกลบินแบบโฮโมไซโกทอาจจะไม่สามารถใช้ประโยชน์จากการเสริมเหล็กได้อย่างเต็มประสิทธิภาพ อย่างไรก็ตาม เมื่อพิจารณาผลการตรวจวัดระดับเฟอร์ไรทินในซีรัมหลังจากการได้รับเหล็กเสริมในกลุ่มหญิงตั้งครรภ์ทั้ง 2 กลุ่ม พบว่า ไม่แตกต่างกัน (ตารางที่ 6) แสดงให้เห็นว่าการได้รับเหล็กเสริมไม่น่าจะมีผลทำให้เกิดภาวะเหล็กเกิน ดังนั้น ถึงแม้ว่าการให้เหล็กเสริมในหญิงตั้งครรภ์ที่มียีนบีตาอีโกลบินอาจจะไม่ส่งผลให้มีการสร้างฮีโมโกลบินได้เพิ่มมากขึ้น แต่การให้เหล็ก

เจริญย่อมมีความจำเป็นสำหรับเทวดาในความซึ่งจำเป็นจะต้องใช้สารคุณเหล็กในการสร้างเม็ดเลือดแดง เช่นเดียวกัน

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OUTPUT ที่ได้จากโครงการ

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ผลงานที่นำเสนอในที่ประชุมวิชากการ

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2. Sanchaisuriya K, Fucharoen S, Fucharoen G, Ratanasiri T, Changtrakun Y, Ukosanakarn U, Ussawaphark W. Effect of thalassemia genes and iron status on the screening of thalassemia and hemoglobinopathies in pregnancy. The 5th Congress of the Royal Golden Jubilee program, 23-25 April 2004, Jom Tein Palm Beach Resort, Pattaya, Chonburi. (Oral presentation)
3. Sanchaisuriya K, Chunpanich S, Fucharoen S, Fucharoen G, Sanchaisuriya P, Changtrakun Y. Association of Hb Q-Thailand with homozygous Hb E and heterozygous Hb Constant Spring in pregnancy. The Xth Congress of the International Society of Hematology, Asian-Pacific Division, 1-5 September 2004, Nagoya, Japan. (Oral presentation)

APPENDIX

ผลงานที่ตีพิมพ์ในวารสารวิชาการ

A Reliable Screening Protocol for Thalassemia and Hemoglobinopathies in Pregnancy

An Alternative Approach to Electronic Blood Cell Counting

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Key Words: Thalassemia screening; α-Thalassemia; β-Thalassemia; Hemoglobin E; Osmotic fragility test, Dichlorophenolindophenol test, RBC indices

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Abstract

Primary screening for thalassemia and hemoglobinopathies usually involves an accurate blood count using an expensive electronic blood cell counter. A cheaper alternative method was tested by using a modified osmotic fragility (OF) test and a modified dichlorophenolindophenol (DCIP) test. Altogether 423 pregnant Thai women participated in this project. Hemoglobin patterns and globin genotypes were determined using an automated high-performance liquid chromatography analyzer and polymerase chain reaction analysis of α- and β-globin genes. Among the 423 subjects, 264 (62.4%) carried thalassemia genes. The combined OF and DCIP tests detected all pregnant carriers of the 3 clinically important thalassemias, ie, α^0-thalassemia, β-thalassemia, and hemoglobin E with a sensitivity of 100.0%, specificity of 87.1%, positive predictive value of 84.5%, and negative predictive value of 100.0%, which show more effectiveness than these values for the standard method based on RBC counts. A combination of modified OF and DCIP tests should prove useful and applicable to prenatal screening programs for thalassemia and hemoglobinopathies in communities with limited facilities and economic resources.

Thalassemia and hemoglobinopathies, the most common inherited disorders of hemoglobin (Hb) synthesis, are among the major public health problems in many areas of the world, including Southeast Asia.[1,2] Although gene-gene interactions in this population can lead to several thalassemia syndromes, 3 targeted for prevention and control measures are homozygous α^0-thalassemia causing the Hb Bart hydrops fetalis, homozygous β-thalassemia, and β-thalassemia/Hb E.[3] Therefore, in a prevention and control program, rapid, accurate, and inexpensive screening protocols to identify carriers of α^0-thalassemia, β-thalassemia, and Hb E, especially in a prenatal population at risk for Hb disorders, are essential.

Conventionally, the primary screening method for all forms of thalassemia relies on hematologic index cutoffs, which involves an accurate blood count using an electronic cell counter. Individuals with mean corpuscular volume (MCV) values less than 80 μm^3 (<80 fL) and mean corpuscular hemoglobin (MCH) values less than 27 pg should be examined further to confirm or exclude the diagnoses of α-thalassemia and β-thalassemia.[4,5] This, however, requires an expensive electronic blood cell counting apparatus and cannot be applied in rural areas where laboratory facilities and economic resources are limited.

It has been demonstrated that a single-tube osmotic fragility (OF) test with 0.36% saline solution might be an attractive alternative to identify carriers of α- and β-thalassemias.[6-8] Recently it was demonstrated that the use of 0.34% instead of 0.36% saline solutions could greatly improve the specificity of the OF test for α^0-thalassemia and β-thalassemia, but an Hb E carrier would be missed.[9] A combined modified OF test and modified dichlorophenolindophenol (DCIP) test for Hb E[10] has been proposed for screening in

rural communities of Southeast Asia.[11] However, this screening protocol might not be appropriate for pregnant women because anemia might be more prevalent and severe owing to physiologic changes and/or iron deficiency. Therefore in this study, the effectiveness of the combined modified OF test and modified DCIP test for the identification of α^0-thalassemia, β-thalassemia, and Hb E in pregnancy was tested and compared with other standard screening protocols involving measurement of RBC indices.

Materials and Methods

Subjects

Study participants included 423 apparently healthy, pregnant Thai women consecutively attending an antenatal care service between January and December 2003 at Nampong and Chumpae district hospitals of Khon Kaen province in northeast Thailand. The study was approved by the institutional ethical committee of Khon Kaen University, Khon Kaen, Thailand. Only women in the first or second trimester were recruited. The mean ± SD age and gestational age of the subjects were 25.5 ± 6.0 years and 12.7 ± 3.7 weeks, respectively. After informed consent was obtained at the first visit to the antenatal care service, EDTA-anticoagulated blood samples were obtained and transferred on ice within 2 hours to the Faculty of Associated Medical Sciences, Khon Kaen University, where all laboratory investigations were performed.

Screening and Hematologic Analysis

Screening for thalassemia was performed with a modified OF test and for Hb E with a modified DCIP test using the KKU-OF and the KKU-DCIP-Clear reagent kits (PCL Holding, Bangkok, Thailand) and following the manufacturer's protocols as described.[11] Briefly, in the OF test, a sample of 20 μL of whole blood was mixed with 2 mL of 0.34% buffered saline solution in the test tube and left at room temperature for 15 minutes before being interpreted. For the DCIP precipitation test, 20 μL of whole blood was added to 2 mL of a modified DCIP reagent, and the mixture was incubated at 37°C for 15 minutes before the addition of 20 μL of stopping reagent supplied by the manufacturer to eliminate and decolorize the excess DCIP dye.

Both tests were interpreted by visualization as negative or positive. Negative samples are characterized by a clear solution and positive samples by a cloudy appearance. RBC indices were determined using the Coulter GenS automated blood cell counter (Coulter Electronics, Hialeah, FL). Hb patterns and levels were determined using an automated high-performance liquid chromatography system (Variant, Bio-Rad Laboratories, Hercules, CA).

DNA Analysis

Genomic DNA was extracted from peripheral blood WBCs by using a standard method. All common α-thalassemia mutations, including α^0-thalassemia (SEA type), α^+-thalassemia (3.7- and 4.2-kilobase deletions), Hb Constant Spring (Hb CS) and Hb Paksé (Hb Ps) were identified by the polymerase chain reaction (PCR) and related methods.[12-15] Common β-thalassemia mutations in Thailand also were examined in samples with Hb A_2 levels exceeding 3.5% using the allele-specific PCR routinely run in our laboratory.[16,17]

Statistical Analysis

Descriptive statistics, including mean and SD, were used to describe hematologic features of the subjects. To compare the effectiveness of the standard method based on RBC indices with that of the combined modified OF and DCIP tests for thalassemia and Hb E screening, we calculated sensitivity, specificity, positive predictive value, and negative predictive value. The results of Hb analysis with high-performance liquid chromatography and PCR analysis of α- and β-thalassemias were used as "gold standards."

Results

The results of thalassemia genotyping and hematologic characteristics of 423 pregnant women are summarized in **Table 1**. Among 423 pregnant women studied, 264 (62.4%) were found to carry thalassemias or hemoglobinopathies. No thalassemia gene was detected in the remaining 159 subjects (37.6%). In the former group, 22 thalassemia genotypes were observed. As expected, the most common genotype was Hb E heterozygote, which was identified in 94 subjects. Interactions of Hb E with several forms of α-thalassemia also were found. α-Thalassemias, including α^0- and α^+-thalassemia, Hb CS, and Hb Ps, were identified as pure heterozygotes or in association with other abnormal genes. Three β-thalassemia heterozygotes were detected. Two as yet undescribed conditions of homozygous Hb E with Hb Q-Thailand and a compound Hb Q-Thailand/Hb CS also were observed, which will be reported elsewhere. Compared with the nonthalassemia group, all forms of thalassemia differed in the RBC indices, particularly the MCV and MCH values. Changes in these parameters clearly were observed in women with α^0- and β-thalassemias and in those who carried 2 or more abnormal genes.

To compare the effectiveness of the screening strategies, the data collected as shown in Table 1 were separated into 2 groups based on genotypes, ie, clinically important and nonclinically important genotypes. The former included α^0-thalassemia, β-thalassemia, and Hb E, which are the target of thalassemia screening.

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Table 1

Thalassemia Genotypes Observed in 423 Pregnant Women and the Corresponding Hematologic Characteristics*

Thalassemia Genotype (No. of Cases)	RBC ($\times 10^{12}$/L)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	Hb Type	HbA_2/E (%)
Heterozygous Hb E (94)	4.6 ± 0.5	11.7 ± 1.1	35.3 ± 3.6	77.5 ± 4.7	25.7 ± 1.8	33.1 ± 1.0	14.3 ± 1.7	EA	29.3 ± 1.7
With α^+-thalassemia (26)†	4.3 ± 0.4	11.6 ± 1.0	35.1 ± 3.1	81.5 ± 3.7	27.1 ± 1.9	33.1 ± 1.1	13.9 ± 1.1	EA	28.0 ± 2.1
With Hb CS (7)	4.1 ± 0.5	11.1 ± 1.1	33.4 ± 3.1	81.9 ± 2.4	27.3 ± 0.9	33.2 ± 0.3	13.7 ± 0.7	EA/CSEA	27.0 ± 1.6
With Hb Ps (3)	5.1 ± 1.0	12.2 ± 1.3	37.9 ± 4.6	74.8 ± 7.7	24.2 ± 2.7	32.4 ± 0.5	15.5 ± 2.5	EA/PsEA	25.7 ± 1.1
With homozygous α^+-thalassemia (2)	5.1, 4.7	12.2, 10.1	37.5, 31.2	72.6, 66.4	23.6, 21.5	32.5, 32.3	15.3, 20.2	EA	21.1, 20.5
With α^0-thalassemia (6)	4.8 ± 0.3	10.5 ± 0.7	32.7 ± 2.0	68.5 ± 4.6	21.9 ± 1.6	32.0 ± 0.7	16.5 ± 1.9	EA	19.6 ± 1.3
With α^+-thalassemia/ Hb Ps (1)	5.01	11.2	34.9	69.6	22.3	32	14.1	EA	20.4
With α^0-thalassemia/ α^+-thalassemia (1)	4.86	7.7	24.7	50.8	15.8	31.1	22.6	EA	15.9
With α^0-thalassemia/ Hb Ps (1)	4.92	7.6	28	56.8	15.5	27.3	23.6	EA	12.6
Homozygous Hb E (16)	4.7 ± 0.5	10.1 ± 0.8	31.4 ± 2.5	66.8 ± 2.6	21.5 ± 0.8	32.2 ± 0.4	16.9 ± 1.0	EE	89.9 ± 4.2
With α^+-thalassemia (3)	4.8 ± 0.5	10.4 ± 0.1	32.1 ± 0.9	67.2 ± 6.4	21.7 ± 1.9	32.4 ± 0.9	16.6 ± 2.4	EE	91.8 ± 4.0
With Hb Q (1)	4.89	10.8	32.7	66.8	22.1	33.1	15.3	EE with Q	79.4
Heterozygous α^0-thalassemia (10)	5.1 ± 0.6	11.0 ± 1.3	34.6 ± 3.9	68.0 ± 2.5	21.6 ± 1.6	31.8 ± 1.5	15.3 ± 1.0	A_2A	2.3 ± 0.3
Heterozygous α^+-thalassemia (53)‡	4.4 ± 0.4	12.1 ± 0.8	36.3 ± 2.6	82.3 ± 3.5	27.4 ± 1.5	33.3 ± 0.9	13.7 ± 1.0	A_2A	2.6 ± 0.3
Heterozygous Hb CS (26)	4.4 ± 0.3	11.6 ± 0.9	35.5 ± 2.9	81.5 ± 5.1	26.7 ± 1.6	32.7 ± 0.8	13.5 ± 0.9	A_2A/CSA_2A	2.6 ± 0.5
Heterozygous Hb Ps (3)	4.4 ± 0.4	12.1 ± 1.1	36.5 ± 4.1	83.4 ± 4.1	27.7 ± 0.7	33.2 ± 0.7	13.3 ± 0.2	A_2A/PsA_2A	2.6 ± 0.2
Homozygous α^+-thalassemia (4)	5.1 ± 0.7	11.7 ± 1.6	35.8 ± 3.4	70.4 ± 2.5	22.8 ± 0.3	32.4 ± 1.4	17.0 ± 2.6	A_2A	2.7 ± 0.5
Compound heterozygous α^0-thalassemia/Hb CS (1)	3.77	7.7	27.8	73.8	20.5	27.8	21.9	CSA_2A Bart Hb	1.0
α^+-thalassemia/Hb Ps (1)	4.81	11.1	35.4	73.6	23.1	31.4	13.9	A_2A	2.1
α^+-thalassemia/Hb CS (1)	5.53	11.9	37.2	67.2	21.5	32	14.5	A_2A	3.1
Hb Q/Hb CS (1)	4.55	9.7	31.6	69.5	21.4	30.7	16.3	A_2A Q	1.5
Heterozygous β-thalassemia (3)	5.1 ± 0.3	10.7 ± 0.9	33.3 ± 2.0	65.5 ± 0.5	21.1 ± 0.5	32.2 ± 0.5	15.7 ± 1.1	A_2A	5.2 ± 0.4
Nonthalassemia (159)	4.1 ± 0.4	12.2 ± 1.1	35.9 ± 4.0	87.9 ± 5.0	29.9 ± 2.1	34.0 ± 1.2	13.7 ± 1.4	A_2A	2.7 ± 0.3

CS, Constant Spring; Hb, hemoglobin; Hct, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Ps, Paksé; RDW, red cell distribution width.

* Values are given as mean ± SD except when n = 1 or n = 2. Values for the RBC count and MCV are given as Système International (SI) units; conversions to conventional units are as follows: RBC count ($\times 10^6$/L), divide by 1.0; MCV (μm^3), divide by 1.0. Other values are given as conventional units; conversions to SI units are as follows: Hb (g/L), multiply by 10.0; Hct (proportion of 1.0), multiply by 0.01; MCH (units are the same); MCHC (g/L), multiply by 10.

† Of the cases, 23 were the $-\alpha^{3.7}$ type, and 3 were the $-\alpha^{4.2}$ type.

‡ Of the cases, 51 were the $-\alpha^{3.7}$ type, and 2 were the $-\alpha^{4.2}$ type.

Table 2 lists the number of women with various genotypes who had positive or negative results based on each screening protocol using standard RBC indices and a combination of modified OF and DCIP tests. Five screening methods were compared, ie, MCV alone (cutoff, 80 μm^3 [80 fL]); MCH alone (cutoff, 27 pg); a combined MCV and MCH; a combined MCV, MCH, and DCIP test; and combined OF and DCIP tests. As shown in Table 2, a total of 126, 138, and 143 women with clinically important thalassemias had positive results for MCV alone, MCH alone, and the combined MCV and MCH, respectively. False-positive and false-negative rates for these 3 screening protocols were 23.6% (39/165) and 19.0% (49/258) for MCV alone, 27.4% (52/190) and 15.9% (37/233) for MCH alone, and 28.5% (57/200) and 14.3% (32/223) for the combined MCV and MCH, respectively.

In contrast with the preceding 3 methods, the combined MCV, MCH, and DCIP protocol and the combined OF and DCIP protocol detected all 175 subjects with clinically important thalassemias. False-positive rates for these 2 screening protocols were 25.2% (59/234) and 15.5% (32/207), respectively. No false-negative results were encountered.

Based on these results, the effectiveness of each screening method for the 423 pregnant Thai women was determined and values compared **Table 3**. Excellent sensitivities were obtained with the last 2 protocols (combined MCV, MCH, and DCIP and combined OF and DCIP tests). The combined OF-DCIP method resulted in better specificity, positive predictive value, and negative predictive value than the other methods.

Discussion

The aim of screening for thalassemia and hemoglobinopathies in Southeast Asia is to offer carrier testing to the population before they have children.[2,3] In the present study, we compared the effectiveness of various carrier screening protocols, including existing protocols and a recently established

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With homozygous α^+-thalassemia (2)	5.1, 4.7	12.2, 10.1	37.5, 31.2	72.6, 66.4	23.6, 21.5	32.5, 32.3	15.3, 20.2	EA	21.1, 20.5
With α^0-thalassemia (6)	4.8 ± 0.3	10.5 ± 0.7	32.7 ± 2.0	68.5 ± 4.6	21.9 ± 1.6	32.0 ± 0.7	16.5 ± 1.9	EA	19.6 ± 1.3
With α^+-thalassemia/ Hb Ps (1)	5.01	11.2	34.9	69.6	22.3	32	14.1	EA	20.4
With α^0-thalassemia/ α^+-thalassemia (1)	4.86	7.7	24.7	50.8	15.8	31.1	22.6	EA	15.9
With α^0-thalassemia/ Hb Ps (1)	4.92	7.6	28	56.8	15.5	27.3	23.6	EA	12.6
Homozygous Hb E (16)	4.7 ± 0.5	10.1 ± 0.8	31.4 ± 2.5	66.8 ± 2.6	21.5 ± 0.8	32.2 ± 0.4	16.9 ± 1.0	EE	89.9 ± 4.2
With α^+-thalassemia (3)	4.8 ± 0.5	10.4 ± 0.1	32.1 ± 0.9	67.2 ± 6.4	21.7 ± 1.9	32.4 ± 0.9	16.6 ± 2.4	EE	91.8 ± 4.0
With Hb Q (1)	4.89	10.8	32.7	66.8	22.1	33.1	15.3	EE with Q	79.4
Heterozygous α^0-thalassemia (10)	5.1 ± 0.6	11.0 ± 1.3	34.6 ± 3.9	68.0 ± 2.5	21.6 ± 1.6	31.8 ± 1.5	15.3 ± 1.0	A_2A	2.3 ± 0.3
Heterozygous α^+-thalassemia (53)‡	4.4 ± 0.4	12.1 ± 0.8	36.3 ± 2.6	82.3 ± 3.5	27.4 ± 1.5	33.3 ± 0.9	13.7 ± 1.0	A_2A	2.6 ± 0.3
Heterozygous Hb CS (26)	4.4 ± 0.3	11.6 ± 0.9	35.5 ± 2.9	81.5 ± 5.1	26.7 ± 1.6	32.7 ± 0.8	13.5 ± 0.9	A_2A/CSA_2A	2.6 ± 0.5
Heterozygous Hb Ps (3)	4.4 ± 0.4	12.1 ± 1.1	36.5 ± 4.1	83.4 ± 4.1	27.7 ± 0.7	33.2 ± 0.7	13.3 ± 0.2	A_2A/PsA_2A	2.6 ± 0.2
Homozygous α^+-thalassemia (4)	5.1 ± 0.7	11.7 ± 1.6	35.8 ± 3.4	70.4 ± 2.5	22.8 ± 0.3	32.4 ± 1.4	17.0 ± 2.6	A_2A	2.7 ± 0.5
Compound heterozygous									
α^0-thalassemia/Hb CS (1)	3.77	7.7	27.8	73.8	20.5	27.8	21.9	CSA_2A Bart Hb	1.0
α^+-thalassemia/Hb Ps (1)	4.81	11.1	35.4	73.6	23.1	31.4	13.9	A_2A	2.1
α^+-thalassemia/Hb CS (1)	5.53	11.9	37.2	67.2	21.5	32	14.5	A_2A	3.1
Hb Q/Hb CS (1)	4.55	9.7	31.6	69.5	21.4	30.7	16.3	A_2A Q	1.5
Heterozygous β-thalassemia (3)	5.1 ± 0.3	10.7 ± 0.9	33.3 ± 2.0	65.5 ± 0.5	21.1 ± 0.5	32.2 ± 0.5	15.7 ± 1.1	A_2A	5.2 ± 0.4
Nonthalassemia (159)	4.1 ± 0.4	12.2 ± 1.1	35.9 ± 4.0	87.9 ± 5.0	29.9 ± 2.1	34.0 ± 1.2	13.7 ± 1.4	A_2A	2.7 ± 0.3

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Discussion

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The α-thalassemias, including α^0, α^+, Hb CS, and Hb Ps, also were prevalent and were detected at different frequencies. The high prevalence of Hb E and α-thalassemia in this group of the Thai population was supported by the observation of various interactions of Hb E with several forms of α-thalassemia (Table 1).

In this group of pregnant Thai women, we observed similar hematologic features associated with α^0-thalassemia, β-thalassemia, homozygous Hb E, pure Hb E heterozygote, and double heterozygote for Hb E/α-thalassemia to those reported in nonpregnant subjects.[14-19] Marked reductions in MCV and MCH values with higher numbers of RBCs were observed in women with α^0-thalassemia, β-thalassemia, and homozygous Hb E. All of these forms of thalassemia, therefore, tested positive when MCV or MCH was used as the primary screening tool (Table 2). It is noteworthy that the combined MCV and MCH method provided better sensitivity than that obtained using the MCV or MCH alone. For Hb E heterozygotes, however, many had normal RBC indices and, therefore, had negative results in MCV and MCH screening (Table 2). The false-negative result with MCV and MCH screening for Hb E carrier is unacceptable, especially when a population screened is known to have a high prevalence of Hb E, as is true for Southeast Asian populations. A reduction in MCV and MCH values also was noted in women with other mild forms of thalassemia, ie, α^+-thalassemia, Hb CS, and Hb Ps, compared with the 159 women without thalassemia. Other investigators found similar results in a Chinese population.[20,21]

The results of the present study indicate that during pregnancy, even mild thalassemia might influence hematologic parameters, leading to a false-positive screening result, when the RBC index is used as the primary screening method. The high false-positive and false-negative rates led to disappointing sensitivity and specificity values for the MCV and MCH screening methods. However, as shown in Table 3, the sensitivity of the MCV and MCH screening protocol was improved greatly (to 100.0%) when it was used in combination with a modified DCIP test because all subjects with Hb E had positive results with this test. Based on the results of our study, we strongly recommend the use of a modified DCIP test in addition to electronic blood cell counting for screening Southeast Asian populations to be able to identify and give proper advice to Hb E carriers.

It is interesting that the best screening result with 100.0% sensitivity, 87.1% specificity, 84.5% positive predictive value, and 100.0% negative predictive value was obtained with a combined OF-DCIP protocol (Table 3). According to these data, no false-negative results for the 3 clinically important forms of thalassemia, ie, α^0-thalassemia, β-thalassemia, and Hb E, could be found with this screening method, but approximately 15% of the positive cases would be false-positives. However, this is not important because the major concern of screening is to avoid false-negative results.[22] A screening method consisting of a combination of modified OF and DCIP tests, therefore, is an attractive screening alternative to an expensive electronic blood cell count. The protocol is simple, reliable, cost-effective, and practical enough to be carried out in a primary health care setting. Indeed, the protocol recently has been applied successfully for prevention and control of severe thalassemia in a provincial hospital in Thailand.[23] Using this method in screening for thalassemia and hemoglobinopathies in pregnant women in Southeast Asian communities should facilitate a prevention and control program of this common genetic disorder[24] in the region.

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Association of Hb Q-Thailand with homozygous Hb E and heterozygous Hb Constant Spring in pregnancy

Sanchaisuriya K, Chunpanich S, Fucharoen S, Fucharoen G, Sanchaisuriya P, Changtrakun Y. Association of Hb Q-Thailand with homozygous Hb E and heterozygous Hb Constant Spring in pregnancy. Eur J Haematol 2005: 74: 221–227. © Blackwell Munksgaard 2005.

Abstract: Hemoglobin (Hb) Q-Thailand [α74(EF3): Asp → His] is an abnormal Hb found mainly in China and South-east Asian countries. Association of the $\alpha^{Q\text{-}Thailand}$ allele with α-thalassemia has important implications in diagnosis. We report the hitherto undescribed conditions of this variant in two unrelated pregnant Thai women. Routine Hb analyses using high-performance liquid chromatography identified abnormal Hb migrating after Hb A_2 in addition to a homozygous Hb E in the proband 1 and to a heterozygous Hb Constant Spring (Hb CS) in the proband 2. Further α-globin gene analysis identified that the variant was caused by the *GAC* to *CAC* mutation at codon 74 of the α1-globin gene corresponding to the Hb Q-Thailand, detected *in cis* to the 4.2 kb deletional α-thalassemia 2 in both cases. Interaction of the $\alpha^{Q\text{-}Thailand}$ with the β^{E} globin chains in the proband 1 leads to a Hb variant, namely the Hb QE. Family study of the proband 1 showed that her non-pregnant sister had the same genotype but her father was a double heterozygote for Hb E and Hb Q-Thailand in whom both Hb Q-Thailand and Hb QE were detected. Genotype–phenotype relationships observed in these families with complex hemoglobinopathies are presented and compared with those of simple homozygote for Hb E, heterozygote for Hb CS and heterozygote for Hb Q-Thailand found in other unrelated subjects. A simple DNA assay based on allele-specific polymerase chain reaction for simultaneous detection of the Hb Q-Thailand mutation and the 4.2 kb deletional α-thalassemia 2 determinant was developed and validated.

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Key words: hemoglobin Q-Thailand; hemoglobin E; hemoglobin QE hemoglobin Constant Spring; α-thalassemia; polymerase chain reaction

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Thalassemia and hemoglobinopathies are very common in South-east Asia including Thailand (1). In addition to the two most common hemoglobin (Hb) variants, Hb E [β26(B8): Glu → Lys] and Hb Constant Spring (Hb CS) [αTerm: TAA → CAA], other abnormal Hbs caused by both α-chain and β-chain variants are occasionally reported (2, 3). Therefore cases with complex phenotypes caused by these gene–gene interactions in the same individuals are not uncommon. Among those variants, Hb Q-Thailand [α74(EF3): Asp → His] has been reported in Chinese and other South-east Asian populations in whom the $\alpha^{Q\text{-}Thailand}$ mutation is always linked to the 4.2 kb deletional α-thalassemia 2 determinant (4–6). Interaction of Hb Q-Thailand with α-thalassemia 1 leads to a clinical phenotype of Hb Q-H disease (7–9). Therefore, interaction of this variant with other forms of thalassemia and hemoglobinopathies has important implications in the diagnosis of thalassemia. Using a combination of hematologic and DNA analyses, we have now characterized the previously undescribed conditions caused by interactions of the Hb Q-Thailand with homozygous Hb E and Hb Q-Thailand with Hb CS detected in two unrelated Thai pregnant women. Hematological parameters of the patients are presented and compared with those of the homozygous Hb E, simple heterozygote for Hb CS and simple heterozygote for Hb Q-Thailand. Analytical

characteristics of a new Hb variant resulting from tetrameric assembly of the $\alpha^{Q\text{-Thailand}}$ with the β^E globin chains ($\alpha_2^Q\beta_2^E$) (namely the Hb QE) are demonstrated and a rapid polymerase chain reaction (PCR) assay for simultaneous detection of the $\alpha^{Q\text{-Thailand}}$ mutation and the 4.2 kb deletional α-thalassemia 2 determinant is described.

Materials and methods

Subjects and hematologic analysis

The two probands (P1 and P2) were pregnant women attending the antenatal care service at Chumpae Hospital, Khon Kaen Province, Thailand. Their gestational ages at the time of investigation were 16 and 15 wk respectively. Both of them had neither history of organomegaly nor blood transfusion. Initially, P1 was diagnosed as a homozygous Hb E with unknown variant Hb and P2 was a carrier of a similar Hb variant. After informed consent was obtained, EDTA blood samples were taken from them and their family members and immediately sent on ice to Khon Kaen University for further analysis. Hematological data was collected on an automated blood cell counter (Coulter Gen S; Coulter Electronics, Hialeah, FL, USA). Hb analysis was carried out by electrophoresis on cellulose acetate (pH 8.6) and by automated high-performance liquid chromatography (HPLC) (Variant™; Bio-Rad Laboratories, Hercules, CA, USA) as shown in Fig. 1.

DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes using the standard method. Identification of α-thalassemia 1 (SEA type), α-thalassemia 2 ($-\alpha^{3.7}$ and $-\alpha^{4.2}$), α^{CS} and $\alpha^{Paksé}$ genes was performed using the PCR described elsewhere (10–12). Selective PCR amplification of the α1 and α2-globin genes and direct DNA sequencing were performed as described previously (13).

Allele-specific PCR for simultaneous detection of $\alpha^{Q\text{-Thailand}}$ and $-a^{4.2}$

To develop a direct detection of the *in cis* linked $\alpha^{Q\text{-Thailand}}$ mutation and $-\alpha^{4.2}$ deletion, a multiplex allele-specific PCR approach was developed as shown in Fig. 2. Allele-specific primer αG20 (5′-CAACGCCGTGGCGCACGTGC-3′) was used with a common primer B (5′-GAGGCCCAA GGGGCAAGAAGCAT-3′) located downstream to produce a 416 bp $\alpha^{Q\text{-Thailand}}$ specific fragment. In the same reaction, two other primers – C (5′-GCTAGAGCATTGGTGGGGTCATGCC-3′) and D (5′-TTCTGACTC TGCCCACAGCCTGA-3′) – were also used to produce a fragment of 1529 bp specific for the 4.2 kb deletional α-thalassemia 2 (11). As an internal control, another two additional primers, γ4 (5′-GGCCTAAAACCA CAGAGAGT-3′) and γ5 (5′-CCAGAAGCGAG TGTGTGGAA-3′) (14) were also included to amplify the 578 bp fragment of the $^G\gamma$-globin gene promoter. The 50 µl PCR reaction mixture contained 0.1 µg DNA, 15 pmol of primers αG20, B, γ4 and γ5, 60 pmol of primers C and D, 200 µM dNTPs and 2.5 unit *Taq* DNA polymerase (Promega, Madison, WI, USA.) in 10 mM Tris–HCl (pH 8.0), 50 mM KCl, 1 mM EDTA, 0.1% Triton X-100, 5% glycerol (v/v), 5% DMSO and 3 mM $MgCl_2$. The amplification reaction was carried out in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus, Norwalk, CT, USA). After initial denaturation at 94°C for 3 min, 35 cycles of the PCR process (94°C for 1 min and 65°C for 1 min 30 s) were performed. The amplified product was analyzed on 1.5% agarose gel electrophoresis and visualized under UV light after staining with ethidium bromide.

Results

Hematological parameters and genotypes of the two probands and their family members are summarized in Table 1. For comparison, additional data from pregnancies with homozygous Hb E, heterozygous Hb CS and non-pregnant subjects with heterozygous Hb Q-Thailand and Hb Q-H disease in our series are also presented. While hematological values of the P1, a homozygous Hb E with Hb variant were similar to those of simple homozygous Hb E, the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and MCH concentration (MCHC) values of P2 were obviously lower than those of the heterozygous Hb CS. As shown in Fig. 1A, Hb electrophoresis of the P1 and her family members demonstrated that she and her sister were both homozygous Hb E with abnormal Hb. This abnormal Hb migrated more slowly than that of the Hb E (Fig. 1A, lanes 4 and 5). Her mother (Fig. 1A, lane 3) was a pure carrier of Hb E whereas her father (Fig. 1A, lane 2) was a carrier of Hb E with two additional abnormal Hb bands, one of which had the same electrophoretic mobility with those detected in P1 and her sister, whereas another migrated more anodic to the Hb E. Hb analysis with an automated HPLC revealed abnormal Hb peaks eluted after Hb E/A_2 in both P1 (Fig. 1B) and P2 (Fig. 1C) with very close retention times, i.e. 4.82 min in the P1 and 4.65 min in the P2. The amounts of these abnormal Hbs were 13.7% and 49.3% in P1 and P2 respectively

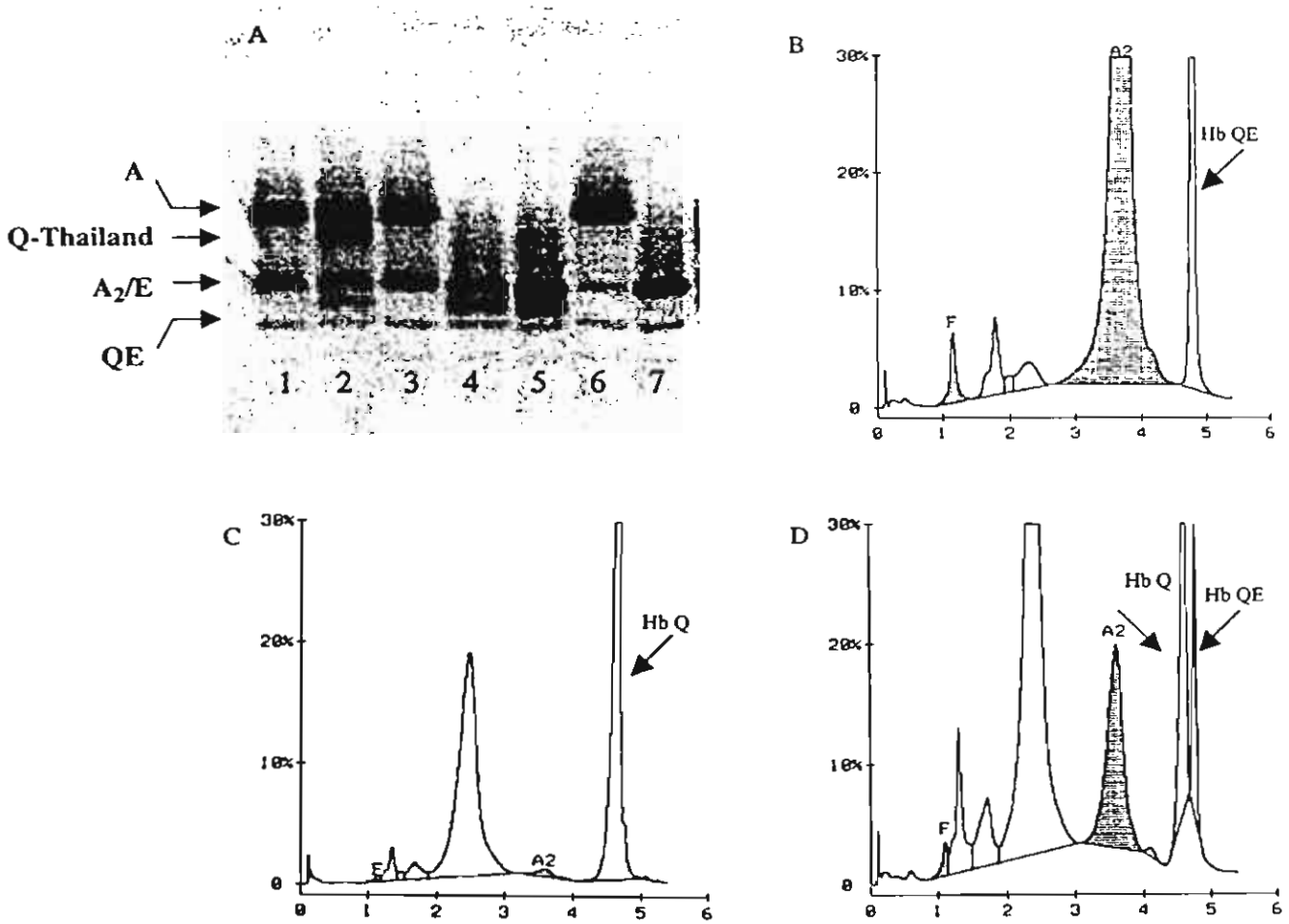

Fig. 1. Hemoglobin analysis of the patients and family members. (A) Cellulose acetate electrophoresis at pH 8.6. Lane 1: heterozygous Hb E, lane 2: P1's father, lane 3: P1's mother, lane 4: P1, lane 5: P1's sister, lane 6: normal individual and lane 7: homozygous Hb E. The positions of Hb Q-Thailand, Hb QE as well as Hb A and Hb A_2/E are indicated. B–D are HPLC profiles of the P1, P2 and P1's father respectively. The elution peaks of Hb Q-Thailand and Hb QE are indicated by arrows.

(Table 1). No Hb E was identified in the P2. HPLC analysis of the P1's father demonstrated in addition to the Hb E heterozygote, two abnormal Hb peaks, quite similar to those identified separately in P1 and P2 with the amounts of 14.4% and 5.7% respectively (Fig. 1D). These data indicate that although the two abnormal Hbs identified in P1 and P2 were very similar, they were not the same variant. As shown in Table 1, although most of the hematologic values of the P1's father were normal, he had relatively lower Hb E level as compared with the P1's mother, a simple carrier of Hb E (19.9% vs. 28.3%), the data indicating a possibility of co-inheritance of α-thalassemia (15, 16). Therefore, α-globin genotyping by PCR was carried out for all individuals. With this PCR analysis, we identified the 4.2 kb deletional α-thalassemia 2 in P1, P1's father, P1's sister and P2 and the Hb CS gene in P2 and P2's father who was a carrier of Hb CS (Table 1). Further analysis by DNA sequencing of the amplified α1 globin genes of the two probands identified the same *GAC* (Asp)–*CAC* (His) mutation at codon 74, corresponding to the Hb Q-Thailand described previously (5, 8). Therefore with this analysis, we were able to conclude that P1 was a homozygous Hb E with Hb Q-Thailand and P2 was a double heterozygote for Hb Q-Thailand and Hb CS, both of which have not previously been encountered. It is conceivable that an abnormal Hb observed in the P2 was the Hb Q-Thailand resulting from a tetrameric assembly of the $\alpha^{Q\text{-Thailand}}$ and β^{A} chains ($\alpha_2^Q\beta_2^A$) whereas that observed in the P1 would be most likely the result of tetrameric assembly of $\alpha^{Q\text{-Thailand}}$ and β^{E} chains ($\alpha_2^Q\beta_2^E$) as P1 has no β^A. The latter interaction would result in a new Hb variant which is named the Hb QE rather than the Hb Q-Thailand described before.

In order to establish a simultaneous and rapid method for detection of a linked Hb Q-Thailand/α-thalassemia 2, a multiplex allele-specific PCR

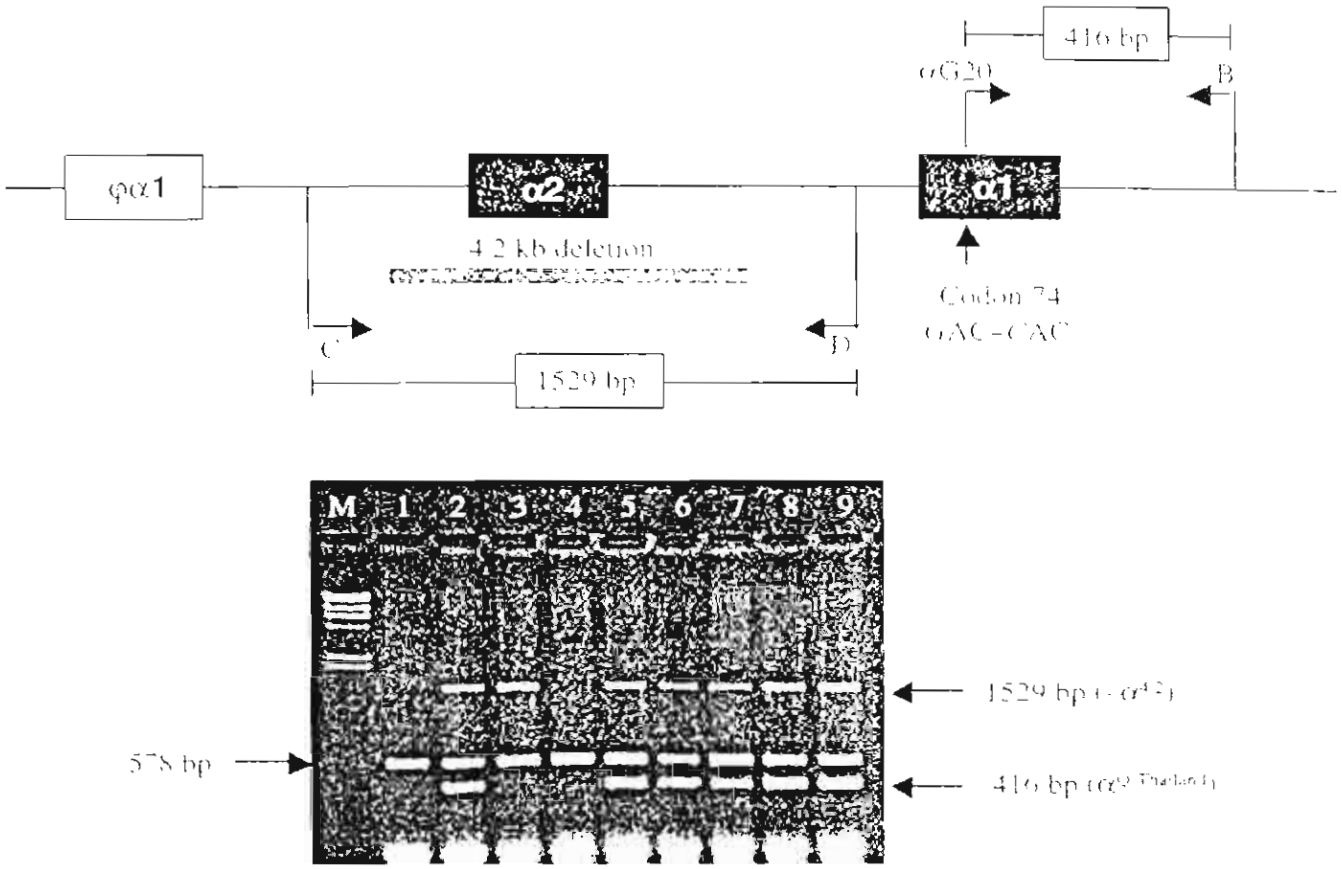

Fig. 2. A multiplex allele-specific PCR assay for simultaneous detection of the $-\alpha^{4.2}$ and the $\alpha^{Q\text{-Thailand}}$ mutations. The locations and orientations of primers C, D, αG20 and B are indicated. The 1529 bp, 578 bp, 416 bp fragments are specific for the $\alpha^{Q\text{-Thailand}}$ and the $-\alpha^{4.2}$ respectively. The 578 bp is an internal control fragment of the $^{G}\gamma$-globin gene promoter. M, ΦX/*Hae* III size markers; 1, normal control; 2, Hb Q-Thailand carrier; 3, $-\alpha^{4.2}$ carrier; 4, P1, $\alpha\alpha/-\alpha^{4.2}$; 5, P1's father; 6, P1; 7, P1's sister; 8, P2 and 9, Hb Q-H disease.

methodology was developed (Fig. 2). Primers C and D were used to identify the 4.2 kb deletional α-thalassemia 2 on an α2-globin gene with the amplified fragment of 1529 bp whereas αG20 and B were specific for the $\alpha^{Q\text{-Thailand}}$ mutation on the *in cis* α1-globin gene with the amplified fragment of 416 bp. Amplification of the 578 bp $^{G}\gamma$-globin gene promoter with primers γ4 and γ5 mentioned in the Materials and methods section was used as an internal control of PCR amplification. A representative agarose gel electrophoresis of this PCR analysis for the P1 family is shown in Fig. 2. While normal control has only one band of 578 bp (Fig. 2, lane 1), a carrier of the 4.2 kb α-thalassemia 2 generates an additional band at 1529 bp (Fig. 2, lane 3). All subjects with either Hb Q-Thailand or Hb QE are positive for both the $-\alpha^{4.2}$ (1529 bp) and the $\alpha^{Q\text{-Thailand}}$ (426 bp) mutations (Fig. 2, lanes 2 and 5–9). Identification of both 1529 and 426 bp fragments in an individual with the Hb Q-H disease who carried a SEA deletional α-thalassemia 1 determinant *in trans* to the Hb Q-Thailand mutation (Fig. 2, lane 9) confirms that the $-\alpha^{4.2}$ and the $\alpha^{Q\text{-Thailand}}$ mutations are linked together on the same chromosome. The method has been applied to characterize abnormal Hb on many samples in our routine laboratory. Five other unrelated carriers of Hb Q-Thailand and a patient with Hb Q-H disease were identified and their hematological data are presented in Table 1.

Discussion

Hb Q-Thailand [α74(EF3)Asp → His] (also known as Hb G-Taichung) is an α-globin chain variant resulting from a point mutation in codon 74 of the α1 globin gene, GAC → CAC that leads to a substitution of histidine for aspartic acid. The $\alpha^{Q\text{-Thailand}}$ gene is strongly linked to a leftward single α-globin gene deletion ($-\alpha^{4.2}$) and has important implications in the identification and diagnosis of hemoglobinopathies. The heterozygous form of Hb Q-Thailand is associated with slight microcytosis due to a linked α-thalassemia 2 determinant.

Table 1. Hematological parameters and genotypes of the probands and their family members as compared with homozygous Hb E (Homo. Hb E), heterozygous Hb CS (Het. Hb CS), heterozygous Hb Q-Thailand (Het. Hb Q) and Hb Q-H disease. Hematological values in parentheses of the probands 1 and 2 were obtained at 10 and 8 months after delivery respectively

Parameters	Family 1				Homo. Hb E (N = 18)[1]	Family 2		Het. Hb CS (N = 26)[1]	Het. Hb Q (N = 5)[3]	Hb Q-H disease (N = 1)[4]
	Proband 1	Father	Mother	Sister		Proband 2[2]	Father			
RBC ($\times 10^{12}$/L)	4.9 (5.5)	4.9	5.7	5.8	4.7 ± 0.5	4.5 (5.1)	4.3	4.4 ± 0.3	5.0 ± 0.3	4.4
Hb (g/dL)	10.8 (11.0)	13.9	14.1	12.8	10.1 ± 0.7	9.7 (9.9)	10.2	11.6 ± 0.9	13.5 ± 1.1	9.7
Hct (%)	32.7 (35.1)	42.6	42.7	39.6	31.5 ± 2.3	31.6 (33.3)	32.6	35.5 ± 2.9	41.5 ± 3.7	34.0
MCV (fl)	66.8 (64.0)	86.7	75.2	68.6	67.1 ± 3.2	69.5 (65.7)	76.3	81.5 ± 5.1	82.3 ± 2.6	77.4
MCH (pg)	21.1 (20.1)	28.4	25.0	22.2	21.6 ± 0.9	21.4 (19.5)	23.9	26.7 ± 1.6	27.0 ± 0.6	22.1
MCHC (g/dL)	33.1 (31.4)	32.7	33.2	32.2	32.2 ± 0.5	30.7 (29.7)	31.2	32.7 ± 0.8	32.6 ± 0.5	28.6
RDW (%)	15.3 (16.0)	13.9	14.1	16.5	16.8 ± 1.2	16.7 (15.9)	18.1	13.5 ± 0.9	n.a.	n.a.
Hb-types	EE with Q	EA with Q	EA	EE with Q	EE	A_2A with Hb Q	CS A_2A	CS A_2A/A_2A	A_2A with Hb Q	QH
Hb A_2/E (%)	79.4	19.9	28.3	70.8	90.3 ± 4.2	1.5	2.0	2.6 ± 0.5	2.2 ± 0.2	n.a
Hb Q-T (%)	–	14.4	–	–	–	49.3	–	–	25.4 ± 0.4	78.8
Hb QE (%)	13.7	5.7	–	14.4	–	–	–	–	–	–
α-Genotype	$-\alpha^{Q}/\alpha\alpha$	$-\alpha^{Q}/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$	$-\alpha^{Q}/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$	$-\alpha^{Q}/\alpha^{CS}\alpha$	$\alpha\alpha/\alpha^{CS}\alpha$	$\alpha\alpha/\alpha^{CS}\alpha$	$-\alpha^{Q}/\alpha\alpha$	$-\alpha^{Q}/-$
β-Genotype	β^{E}/β^{E}	β^{E}/β^{A}	β^{E}/β^{A}	β^{E}/β^{E}	β^{E}/β^{E}	β^{A}/β^{A}	β^{A}/β^{A}	β^{A}/β^{A}	β^{A}/β^{A}	β^{A}/β^{A}

[1]All were pregnancies.
[2]Serum ferritin levels at the time of investigation and 8 months after delivery were 2.2 and 247 ng/mL respectively.
[3]Non-pregnant subjects composed of three males and two females.
[4]A 9-yr-old boy.
Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin, MCHC, MCH concentration; RDW, red blood cell distribution width; n.a., not available.

Co-inheritance of Hb Q-Thailand with α-thalassemia 1 results in a thalassemia intermedia known as the Hb Q-H disease with a similar clinical features to that of the deletional Hb H disease, although Hb A is absent in the former (7–9).

We report the hitherto undescribed conditions resulting from the interaction of this abnormal Hb with homozygous Hb E and with heterozygous Hb CS in pregnant Thai women. Although, interaction of Hb Q-Thailand and Hb E heterozygote has been documented pre-natally by DNA analysis in a fetus of a Singaporean family, no protein analysis data were provided (17). We have now demonstrated that interaction of these two Hb variants could lead to another abnormal Hb. The P1 and her sister were both homozygous Hb E with Hb Q-Thailand. As shown in Table 1, they both had hypochromic microcytic red blood cells with no Hb A; however, assembly of the $\alpha^{Q\text{-Thailand}}$ and the β^{E} chains in these two individuals led to the formation of another variant, the Hb QE ($\alpha_2^{Q}\beta_2^{E}$) instead of the Hb Q-Thailand ($\alpha_2^{Q}\beta_2^{A}$) usually encountered. As shown in Fig. 1, these two Hb variants have difference electrophoretic mobilities and HPLC profiles as well as the amounts detectable in blood. The proportions of Hb QE observed in our two cases were relatively low (13.7% and 14.4%) as compared with the level of Hb Q-Thailand observed in five other heterozygotes (25.4 ± 0.4%) (Table 1). The lower proportion of Hb QE as compared with Hb Q-Thailand likely indicates a lower affinity of the $\alpha^{Q\text{-Thailand}}$ chain for the β^{E} chain as compared with the β^{A}-chain in generating Hb molecule. The result of Hb analysis of the P1's father, a double Hb E/Hb Q-Thailand (Fig. 1D) indirectly supports this as he had 14.4% of Hb Q-Thailand ($\alpha_2^{Q}\beta_2^{A}$) and only 5.7% of Hb QE ($\alpha_2^{Q}\beta_2^{E}$). Although, he had apparently lower Hb E level (19.9%) as compared with his wife who was a pure carrier of Hb E (28.3%), his total Hb E level should be in fact 25.6% (i.e 19.9% Hb E + 5.7% Hb QE) which is in the range usually observed for the Hb E heterozygote with α-thalassemia 2 (15, 16). Hematologically, we observed no difference in the hematological values of the P1 and her sister with those encountered in pregnancies with homozygous Hb E (Table 1) although P1 had a relatively lower Hb level as compared with her sister. This could be due to the effect of pregnancy. The same finding was noted for the association of Hb Q-Thailand with heterozygous Hb E observed in her father who had even higher MCV as compared with the simple heterozygous state of Hb E in the mother. These results indicate that co-existence of Hb Q-Thailand with homozygous or heterozygous Hb E does not contribute further to the severity of anemia in the patient. The clinical phenotypic expression observed suggests that both Hb Q-Thailand and its derivative, the Hb QE, share similar functional properties with Hb A.

Hematological analysis of the P2, a compound Hb CS/Hb Q-Thailand showed more hypochromic microcytic anemia as compared with the simple heterozygous Hb CS (Table 1). Although, the markedly reduced MCV and MCH values could result from three α-globin gene defects including the $-\alpha^{4.2}$, the α^{CS} and the $\alpha^{Q\text{-Thailand}}$ with iron deficiency (serum ferritin level; 2.2 ng/mL), we

observed very similar hematological parameters at 8 months after delivery when she had normal serum ferritin level (247 ng/mL). Identification of the α^{CS} mutation without Hb CS in peripheral blood of the patient was not unexpected. The instability of the α^{CS} mRNA and Hb CS molecule can lead to a possibility of misdiagnosis of the Hb CS in routine Hb analysis, unless DNA analysis is performed (15, 16, 18). We therefore recommend DNA analyses of both α- and β-globin genes in all cases with complex thalassemia syndrome to obtain correct genotypes of the patients. With this complex hemoglobinopathy, the P2 would have only one instead of the normally present four α-globin genes. This phenotypic expression was consistent with that of a compound heterozygote for α-thalassemia 2 and Hb CS observed in our series (15). It is also noteworthy that as compared with the other five unrelated carriers of Hb Q-Thailand, P2 had a higher proportion of Hb Q-Thailand (49.3% vs. 25.4 ± 0.4%). This higher proportion of Hb Q-Thailand in this complex hemoglobinopathy should most likely be related to the effect of α-globin gene dosage and the ability to produce Hb A from the intact α-globin genes. In a simple heterozygous state of Hb Q-Thailand, there are two functional α-globin genes *in trans* to the Hb Q-Thailand conferring chromosome. This should result in a higher production of Hb A as compared with that of the P2 who had only one normal α-globin gene and Hb Q-Thailand is consequently reduced.

Nonetheless, this unusually high value of Hb Q-Thailand could lead to a false prediction of a β-chain variant and inappropriate counseling unless DNA analysis is performed. Many Hb variants with similar electrophoretic mobilities or HPLC profiles with those of Hb Q-Thailand and Hb QE have been reported in Asian populations e.g. Hb Tak, Hb D-Punjab, Hb S, Hb Korle-Bu, Hb Siam and Hb G-Makassar (17–24). Interaction of these abnormal Hbs with other hemoglobinopathies and thalassemias can cause serious conditions (25). It is therefore important to distinguish these Hb variants from other α and β chain variants with less or no clinical significance. A simple and rapid method based on the allele-specific PCR shown in Fig. 2 would be an attractive diagnostic alternative for Hb Q-Thailand. Unlike that previously described using *Apa*LI and *Hgi*AI restriction analysis (5), this method does not require additional steps of enzymatic digestion and could identify simultaneously the $\alpha^{Q\text{-}Thailand}$ mutation as well as a linked deletional α-thalassemia 2 (4.2 kb deletion) from a single PCR reaction. The effectiveness of this multiplex PCR approach could favor its application for the definitive diagnosis of Hb Q-Thailand and Hb QE in the population and should facilitate a prevention and control program of hemoglobinopathy in the region.

Acknowledgements

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Nonetheless, this unusually high value of Hb Q-Thailand could lead to a false prediction of a β-chain variant and inappropriate counseling unless DNA analysis is performed. Many Hb variants with similar electrophoretic mobilities or HPLC profiles with those of Hb Q-Thailand and Hb QE have been reported in Asian populations e.g. Hb Tak, Hb D-Punjab, Hb S, Hb Korle-Bu, Hb Siam and Hb G-Makassar (17–24). Interaction of these abnormal Hbs with other hemoglobinopathies and thalassemias can cause serious conditions (25). It is therefore important to distinguish these Hb variants from other α and β chain variants with less or no clinical significance. A simple and rapid method based on the allele-specific PCR shown in Fig. 2 would be an attractive diagnostic alternative for Hb Q-Thailand. Unlike that previously described using *Apa*LI and *Hgi*AI restriction analysis (5), this method does not require additional steps of enzymatic digestion and could identify simultaneously the $\alpha^{Q\text{-}Thailand}$ mutation as well as a linked deletional α-thalassemia 2 (4.2 kb deletion) from a single PCR reaction. The effectiveness of this multiplex PCR approach could favor its application for the definitive diagnosis of Hb Q-Thailand and Hb QE in the population and should facilitate a prevention and control program of hemoglobinopathy in the region.

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ผลงานที่นำเสนอในที่ประชุมวิชาการ

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CITTA' DEL MARE,
TERRASINI, PALERMO - ITALY
OCTOBER 15-19, 2003

PREVALENCE SURVEY AND HEMATOLOGICAL CHARACTERIZATION OF THALASSEMIA AND IRON DEFICIENCY IN THAI PREGNANT WOMEN ATTENDING ANC AT COMMUNITY HOSPITALS

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OBJECTIVE

To determine the prevalence of thalassemia (including Hb E), iron deficiency and the concomitance of thalassemia with iron deficiency in Thai pregnant women and their hematological characteristics.

METHODS

Two hundred and forty-seven Thai pregnant women attending ANC at Nampong and Chumpae hospitals, Khon Kaen province were recruited in the study. Hematological parameters were determined using automated blood cell counter (Coulter STKS, USA.). DNA samples were analysed for α^{o}-thalassemia (SEA-type), α^{+}-thalassemia (3.7 or 4.2 kb deletion), α^{CS}- and α^{Pakse}-gene using PCR and related methodologies. Serum ferritin levels were determined by the ELISA method.

FINDINGS

As high as 59.9 % (148/247) of thalassemia with 20 genotypes was observed. The prevalence of iron deficiency (ferritin level < 20 ug/l) was 13.6% whereas the prevalence of the concomitance of thalassemia with iron deficiency was 6.3%. Of the 240 pregnancies, 59 were anemic (Hb < 11 g/dl). Among them, 86.4% (51/59) carried at least one thalassemia gene. The prevalence of iron deficiency in this group was 9.1%, whereas the concomitance of thalassemia and iron deficiency was 27.3%. Pregnancies who carried thalassemia gene were 6.5 times (95% CI : 3.0-14.3) more likely to have anemia than those who had no thalassemia gene whereas that of iron deficiency were 3.2 times (95% CI : 1.5-6.9) compared to those who had no iron deficiency. Concomitance of Hb E with iron deficiency seemed to have no effect on Hb E levels.

CONCLUSION

Thalassemia and iron deficiency are prevalent and play a major role on anemia in Thai pregnant women. Because of a high prevalence of the concomitance of thalassemia and iron deficiency, it is difficult to use hematological parameters for differentiation between thalassemia and iron deficiency in this population.

RGJ - Ph.D. Congress V

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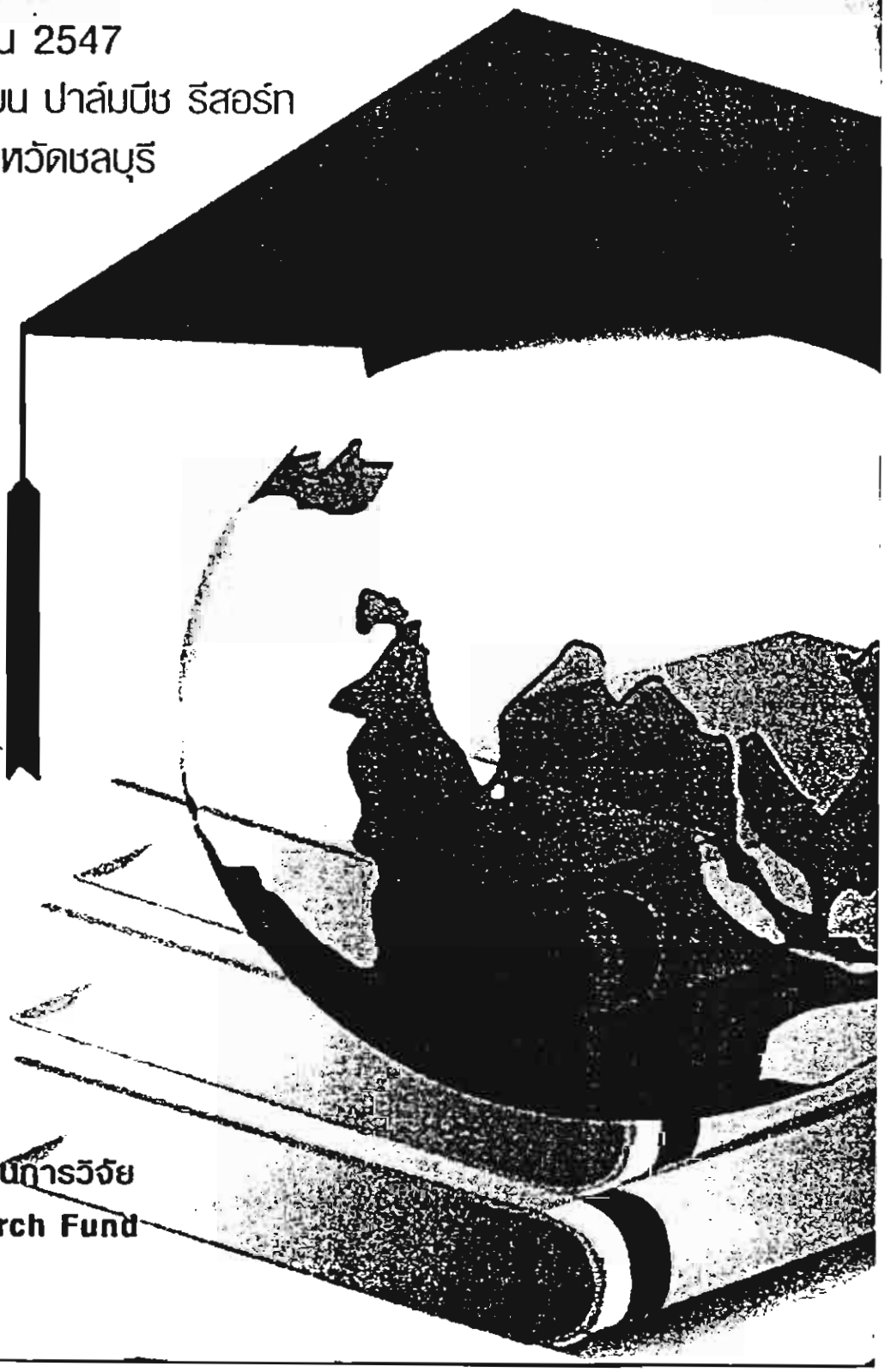

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Effect of thalassemia genes and iron status on the screening of thalassemia and hemoglobinopathies in pregnancy

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Objective

To determine the effect of thalassemia genes and iron status on the screening of thalassemia and hemoglobinopathies in pregnancy.

Methods

Three hundred and eighty-eight blood samples taken from Thai pregnant women were screened for thalassemia and Hb E using a combination of a modified OF test and a modified DCIP test. Serum ferritin levels were determined by an ELISA technique. Erythrocyte indices were investigated using an automated blood cell counter (Coulter GenS: Coulter Electronics, USA.). The results of screening were evaluated with standard hemoglobin analysis using an automated HPLC (Varian: Bio Rad Laboratories, USA.) and PCR analysis of α- and β-globin genes.

Results

Among the 388 pregnant women investigated, 239 (61.6%) were found to carry thalassemia genes with 25 different genotypes, whereas 50 (12.9%) were iron-deficient (ID) pregnancies based on serum ferritin cut-off value of < 15 ug/l. The frequency distributions of iron deficiency in non-thalassemia and thalassemia pregnancies were similar. According to thalassemia screening, the majority (54%) of ID pregnancies showed a negative result while all targeted thalassemia carriers, i.e. α^0-thalassemia, β-thalassemia and Hb E, showed positive results. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the combination of a modified OF test and a modified DCIP test were 100%, 88.6%, 85.9% and 100%, respectively.

Conclusion

A combination of a modified OF test and a modified DCIP test could be used as an effective primary screening for α^0-thalassemia, β-thalassemia and Hb E carriers in pregnancy. Iron deficiency seems to have no effect on the result of screening test. These results ensure a usefulness of the combined screening tests for prevention and control of thalassemia and hemoglobinopathies of the country and other Southeast-Asian countries where laboratory facilities and economic resources are limited.

Keywords: thalassemia, screening, iron status, pregnancy

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Abstract

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O-13

Association of Hb Q-Thailand with homozygous Hb E and heterozygous Hb Constant Spring in pregnancy

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Hb Q-Thailand [α74(EF3): Asp→His] is an abnormal hemoglobin found occasionally in Thailand, China and other Southeast Asian countries. Association of $\alpha^{-3.7}$ gene with α-thalassemia phenotype has important implications in the diagnosis of thalassemia. In this study, interactions of Hb Q Thailand with homozygous Hb E and heterozygous Hb Constant Spring (Hb CS) in two unrelated Thai pregnant women are reported. Hb analysis determined by both cellulose acetate electrophoresis and HPLC revealed additional abnormal hemoglobin migrated after Hb A2/E with the levels of 13.7% in the former and 49.3% in the latter. Molecular analysis of an α2-globin gene showed a GAC→CAC mutation at codon 74 in *cis* to a -4.2 kb deletion α-thalassemia 2. Hematological characteristics of the probands and their families are demonstrated and compared to those of subjects without Hb Q Thailand. A simultaneous simple and rapid PCR assay based on the allele specific polymerase chain reaction (ASPCR) for detection of Hb Q-Thailand and the -4.2 kb deletion α-thalassemia 2 was developed. The method was verified in 3 members of the probands family, 8 additional unrelated subjects with this abnormal hemoglobin and 20 normal individuals. This method should prove useful in complementing routine Hb analysis for definitive diagnosis of this hemoglobinopathy and should facilitate thalassemia prevention and control program in the region.

O-14

Complex interaction of Hb Beijing [alpha 16(A14) Lys-Asn] and Hb L [beta 26(B8) Glu-Lys] with a deletional alpha-thalassemia 1 in a Thai patient.

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Hb Beijing [a 16(A14) Lys→Asn] , a rare fast moving a -chain variant has previously been described in a Chinese whereas Hb E [b 26(B8) Glu→Lys] is the most common b-chain variant among Southeast Asian. We report a hitherto undescribed condition in which these two variants co-segregate in a patient with a-thalassemia. The proband was a 14 yr-old Thai boy who presented with severe hypochromic microcytic anemia with Hb 7.0 g/dl, hematocrit 23.6 %, MCV 47.4 fl, MCH 14.0 pg and MCHC 29.5 g/dl. Routine cellulose acetate electrophoresis at pH 8.6 demonstrated two abnormal Hb bands migrating between Hb A and Hb H and between Hb A and Hb E. HPLC analysis identified a major abnormal peak at the J-window (39.1%) and two minor peaks at the Hb A and Hb E positions with the amounts of 10.3 % and 14.8 %, respectively, indicating a compound heterozygosity for Hb E and an unknown a -chain variant. Further DNA analysis of the proband revealed a AAG-AAT mutation at codon 16 of the a 2-globin gene corresponding to the Hb Beijing mutation in trans to the SEA deletional a thalassemia 1. He was therefore a triple heterozygote for Hb Beijing/Hb E/a-thalassemia 1. Family study indicated that his father was a double heterozygote for Hb Beijing and Hb E without a -thalassemia whereas his mother was a simple carrier of a -thalassemia 1. The genotype-phenotype relationship observed in this Thai family with complex hemoglobinopathies is presented and a simple DNA assay based on the allele-specific PCR approach for detection of Hb Beijing is demonstrated.