รายงานวิจัยฉบับสมบูรณ์

โครงการ 1. ปัญหาและการป้องกันไวรัสตับอักเสบในประเทศไทย

2. อณูชีววิทยาทางการแพทย์

ศาสตราจารย์นายแพทย์ยง ภู่วรวรรณ และคณะ

กรกฎาคม 2547

สัญญาเลขที่ RTA/03/2544

รายงานวิจัยฉบับสมบูรณ์

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คณะผู้วิจัย		สังกัด
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18. อ.ดร.จินตนา	จิรถาวร	คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
19. อ.ดร.ปิยะศักดิ์	ชะอุ่มพฤกษ์	คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
20. ผศ.ดร.วนิดา	นพพรพันธุ์	คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
21. รศ.พญ.ดร.สุรางค์	ไตรธีระประภาพ	คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
22. รศ.น.สพ.ดร.คณิศักดิ์	อรวีระกุล	คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
23. ศ.ท.พญ.กอบกาญจน์	ทองประสม	คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
24. นางสาวอภิรดี	เทียมบุญเลิศ	โรงพยาบาลจุฬาลงกรณ์ สภากาชาดไทย
25. นพ.ศุภมิตร	ชุณห์สิทธิวัฒน์	กระทรวงสาธารณสุข

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

โครงการ 1. ปัญหาและการป้องกันไวรัสตับอักเสบในประเทศไทย

2. อณูชีววิทยาทางการแพทย์

การศึกษาวิจัยในกลุ่มเมธีวิจัยอาวุโส สกว. ศ.นพ.ยง ภู่วรวรรณ ครั้งที่ 2 ในช่วงระยะเวลา 3 ปี ตั้งแต่ 1 สิงหาคม 2544 – 31 กรกฎาคม 2547 โดยทำการศึกษาวิจัยทั้งทางวิทยาศาสตร์พื้นฐานและวิจัยคลินิกเกี่ยวกับโรคไวรัสตับอักเสบ เอ บี ซี ดี อี จี ทีที และเซนไวรัส อณูชีววิทยาทางการแพทย์และไวรัสที่เป็นปัญหาในประเทศไทย มีผลงานเกิดขึ้น สามารถประยุกต์ไปใช้เป็นประโยชน์สำหรับประเทศไทยทั้งทางด้านระบาดวิทยา การติดตามดำเนินโรค การรักษาและการป้องกัน ผลงานที่เกิดขึ้นได้รับการลงพิมพ์ในวารสารระดับนานาชาติที่อยู่บนฐานข้อมูล PubMed หรือ Medline มากกว่า 50 เรื่อง มีนิสิตปริญญาดุษฎีบัณฑิตจบการศึกษาแล้ว 2 คน และนิสิตมหาบัณฑิตจบการศึกษาแล้วมากกว่า 10 คน

งานวิจัยด้านไวรัสตับอักเสบที่เป็นปัญหาในประเทศไทย เรียงตามลำดับความสำคัญจากไวรัสตับอักเสบ บี ซี และ เอ

ไวรัสตับอักเสบ บี

ในปัจจุบันได้มีการป้องกันโดยการให้วัคซีนกับทารกแรกเกิดทุกคน ตั้งแต่ปี พ.ศ. 2531 เป็นต้นมา จากการศึกษาในปี 2542-2543 พบว่าเด็กที่มีอายุน้อยกว่า 10 ปี เป็นพาหะเหลือเพียงร้อยละ 0.7 และเด็กอายุ 10-18 ปี เป็นพาหะร้อยละ 3.5 (เดิมเด็กไทยเป็นพาหะร้อยละ 5-6) ลดลงอย่างมาก ขณะนี้ทางกลุ่มร่วมกับกรมควบคุมโรค กระทรวงสาธารณสุข กำลังดำเนินการศึกษาถึงผลกระทบการฉีดวัคซีนป้องกันในทารก โดยทำการศึกษาใน 4 จังหวัด ได้แก่ จังหวัดชลบุรี จังหวัดเชียงราย จังหวัดอุดรธานี และจังหวัดนครศรีธรรมราช จำนวน 6,000 คน และจะทำการศึกษาถึงข้อมูลของไวรัสตับอักเสบ เอ และ ซี ด้วย คาดว่าการศึกษาจะแล้วเสร็จภายในสิ้นปี 2547 นี้

การให้วัคซีนมาเป็นระยะเวลานานกว่า 10 ปี ในปัจจุบันเป็นปัญหาว่าจำเป็นต้องกระตุ้นอีกครั้งหลังให้วัคซีนนานเกิน 5 ปี หรือไม่ จากข้อมูลการศึกษาผลของวัคซีนระยะยาวจนถึงปีที่ 15 พบว่ายังไม่มีความจำเป็นในการกระตุ้นวัคซีนป้องกันไวรัสตับอักเสบ บี ผลการศึกษาระยะยาว 15-20 ปี กำลังอยู่ในระหว่างการศึกษาติดตามในเด็กประมาณ 200 คน ผลการศึกษาดังกล่าวจะเป็นการศึกษาติดตามผลของการฉีดวัคซีนในทารกกลุ่มเสี่ยงที่ยาวที่สุดในโลกและข้อมูลจะเป็นประโยชน์อย่างมากในการป้องกันการติดเชื้อไวรัสตับอักเสบ บี

การศึกษาไวรัสตับอักเสบ บี ในแนวลึกทางอณูชีววิทยา ทางกลุ่มได้ทำการศึกษาสายพันธุ์และพัฒนาวิธีการตรวจแยกสายพันธุ์ genotype และวัดเชิงปริมาณโดยใช้ melting curve analysis ของ Real Time PCR ได้ผลดียิ่ง และชนิดของ genotype ยังมีความสำคัญทางคลินิก และการดำเนิน

โรคในผู้ป่วยไวรัสตับอักเสบ บี กลุ่มยังได้ทำการศึกษาลักษณะการกลายพันธุ์ชนิดต่าง ๆ ของไวรัสตับอักเสบ บี และความสำคัญทางคลินิกอีกด้วย

การศึกษาไวรัสตับอักเสบ บี ในชะนีทำให้ทราบลักษณะสายพันธ์อุบัติการณ์และการติดต่อจากแม่ลูกชะนี ข้อมูลสำคัญที่จะศึกษาต่อและวางแผนป้องกันที่จะติดต่อมาสู่คน

ไวรัสตับอักเสบ ซี

ในช่วง 3 ปี ที่ผ่านมา กลุ่มวิจัยได้ทำการศึกษาระบาดวิทยาอุบัติการณ์ของกลุ่มเสี่ยงเช่น ในกลุ่มติดยาเสพติดชนิดฉีดเปรียบเทียบกับกลุ่มบริจาคโลหิต พบว่ากลุ่มติดยาเสพติดชนิดฉีดมีอุบัติการณ์การติดเชื้อไวรัสตับอักเสบ ซี เกิดขึ้นเร็วมาก ภายใน 6 เดือนถึง 1 ปี ผู้ติดยาเสพติดชนิดฉีดมากกว่าร้อยละ 80 จะติดเชื้อไวรัสตับอักเสบ ซี โดยมากเมื่อติดเชื้อแล้วจะเรื้อรัง โดยในระยะแรกจะไม่มีอาการของโรค กลุ่มวิจัยยังได้ทำการศึกษาการติดเชื้อเฉียบพลันโดยเฉพาะที่ติดจากการได้รับโลหิต โดยพิสูจน์การจำแนกสายพันธุ์ของผู้ให้และผู้รับทางวิธีการอณูชีววิทยา

การศึกษาระบาดวิทยา genotype ของไวรัสตับอักเสบ ซี ในประเทศไทย ที่พบบ่อยเป็น genotype 3a, 1b และ 6v โดยที่ genotype 3a จะให้ผลดีต่อการรักษาด้วย interferon นอกจากนี้ทางกลุ่มได้ทำการศึกษาเปรียบเทียบกับการตรวจ genoype ด้วยวิธีต่างๆ พบว่าการตรวจโดยวิธีการถอดรหัสจะให้ผลดีและถูกต้องมากที่สุด นอกจากนี้ยังได้ศึกษาถึงความสำคัญของการติดเชื้อในคู่สามีภรรยา โดยพบว่าโอกาสติดเชื้อเกิดขึ้นได้น้อยมาก

การศึกษาถึงระบบภูมิต้านทานต่อไวรัสตับอักเสบ ซี เพื่อจะได้ใช้เป็นพื้นฐานของการที่จะนำวัคซีนในอนาคตมาใช้ ยังอยู่ในระหว่างการดำเนินการและยังได้ร่วมมือทำการศึกษาวิจัยไวรัสตับอักเสบ ซี กับประเทศเนเธอร์แลนด์ และญี่ปุ่นอีกด้วย

ไวรัสตับอักเสบ เอ

ข้อมูลการศึกษาของกลุ่มมีเป็นจำนวนมากที่สุดในประเทศไทยใช้เป็นข้อมูลในการวางแผนการป้องกันโดยเฉพาะการป้องกันด้วยวัคซีน การศึกษาระบาดวิทยาทำให้เป็นที่ยอมรับกันว่าประเทศไทยมีความชุกชุมของโรคไวรัสตับอักเสบ เอ ลดลงอย่างมาก อย่างไรก็ตาม โรคดังกล่าวยังมีการระบาดเกิดขึ้นได้ โดยเฉพาะในสถานเลี้ยงเด็กที่มีเด็กอยู่ร่วมกันเป็นจำนวนมากและในโรงเรียน กลุ่มวิจัยได้ทำการศึกษาการระบาด 2 แห่ง คือ ที่จังหวัดนราธิวาส อำเภอรือเสาะ และสถานเลี้ยงเด็กกำพร้า ทำให้ทราบถึงลักษณะการระบาดรวมทั้งอัตราการเกิดโรคหลังจากได้รับหรือติดเชื้อ

การศึกษาในแนวลึกได้ทำการจำแนกสายพันธุ์ของไวรัสตับอักเสบ เอ ที่เกิดขึ้นในประเทศไทย พบว่าเป็น genotype 1A

นอกจากไวรัสตับอักเสบ เอ บี และ ซี แล้ว ทางกลุ่มวิจัยได้ทำการศึกษาถึงไวรัสตับอักเสบ ดี พบปัญหาของไวรัสตับอักเสบ ดี อยู่ในกลุ่มผู้ติดยาเสพติดชนิดฉีดเท่านั้นและจำแนกสายพันธุ์ได้เป็น genotype 1 เหมือนกับการติดเชื้อในประเทศยุโรปและอเมริกา

ไวรัสตับอักเสบ อี ในผู้ป่วยตับวาย พบว่าไม่เป็นปัญหาในประเทศไทย ทางศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบมีข้อมูลไวรัสตับอักเสบ จี และทีที ซึ่งในประเทศไทยพบในบุคคลทั่วไปเช่น ผู้บริจาคโลหิตและสตรีตั้งครรภ์ร้อยละ 5 และ 7 ตามลำดับ พบได้บ่อยในกลุ่มเสี่ยงต่างๆ ด้วย

สำหรับ SEN ไวรัส เป็นไวรัสที่พบใหม่และเริ่มมีรายงานตั้งแต่ปี 2543 ทางกลุ่มได้ทำการศึกษาไวรัสดังกล่าว พบได้ในโรคตับเรื้อรัง ตับแข็งและมะเร็งตับสูงกว่าคนปกติ แต่อย่างไรก็ตามการศึกษาใน case control study ไม่พบเป็นปัจจัยเพิ่มขึ้นในการทำให้เกิดโรคตับเรื้อรัง ตับแข็ง SEN ไวรัสที่พบในประเทศไทยและทำการศึกษาไว้คือ SENV-D และ SEN-H

ทางกลุ่มวิจัยยังได้ใช้วิธีการทางอณูชีววิทยาทางการแพทย์ในการศึกษาวินิจฉัยไวรัสชนิดอื่นๆ ในประเทศไทยอีก เช่น การศึกษา human metapneumovirus ในผู้ป่วยเด็กปอดอักเสบในประเทศไทย ทำให้สามารถวินิจฉัยและทราบอุบัติการณ์ รวมทั้งทำการศึกษาถึงไวรัสที่ทำให้เกิดโรคทางเดินหายใจชนิดอื่นๆ ในประเทศไทยอีกด้วย เช่น Respiratory syncytial virus (มี 2 subtype คือ subtype A และ B), coronavirus ชนิด 229E และ OC 43

ไวรัส Rota เป็นปัญหาสำคัญให้เกิดโรคอุจจาระร่วงในเด็กและเป็นปัญหาสำคัญทางสาธารณสุขมาโดยตลอด จะพบผู้ป่วยมากในฤดูหนาว ในปัจจุบันวัคซีนที่ใช้ป้องกันโรคยังอยู่ในระหว่างการศึกษาวิจัยในระยะสุดท้ายในประเทศต่างๆ รวมทั้งประเทศไทย และแต่เดิมทราบว่าสายพันธุ์ที่พบในประเทศไทยเป็น genotype G1 (จากการศึกษาโดยกลุ่มวิจัยที่ได้รับทุนเมธีวิจัยครั้งที่ 1) แต่จากการศึกษาใหม่ใน 2 ปีที่ผ่านมาพบว่าการระบาดในประเทศไทยเป็นสายพันธุ์ G2 และ G9 ดังนั้น วัคซีนที่จะนำมาใช้ในอนาคตต้องสามารถป้องกันสายพันธุ์ G9 ได้ด้วย

ไวรัส Norwalk ทำให้เกิดโรคอุจจาระร่วงได้ในเด็กและผู้ใหญ่ ทางกลุ่มได้พัฒนาการตรวจวินิจฉัยและทำให้ทราบอุบัติการณ์ของโรค โดยเฉพาะเด็กที่มีโรคอุจจาระร่วงนับเป็นการเพิ่มศักยภาพในการตรวจวินิจฉัยโรคดังกล่าวเกิดขึ้นในประเทศไทย

ไวรัสในกลุ่ม Herpes 1-8 ทางกลุ่มได้ให้ความสนใจและได้ทำการศึกษาในการพัฒนาการตรวจวินิจฉัยจำแนกแยกชนิด แยกใช้วิธี multiplex PCR และ consensus PCR ร่วมกับ RFLP ทำให้เพิ่มประสิทธิภาพในการวินิจฉัยและแยกชนิดของไวรัสได้เป็นอย่างดี รวมทั้งทางกลุ่มยังให้ความสนใจในการวินิจฉัย Herpes ไวรัสใน nonhuman primate เช่นรายงาน Herpes virus ในลิงอุรังอุตังในวารสาร J Med Primatology และอยู่ระหว่างการศึกษา lymphocryptovirus ในชะนี โดยนิสิตปริญญาโทอีกด้วย (อยู่ระหว่างการศึกษา)

ไวรัส Parvo B19 เป็นไวรัสอีกตัวหนึ่งที่ทางกลุ่มให้ความสนใจและสามารถพัฒนาการตรวจวินิจฉัยได้อย่างมีประสิทธิภาพ ปัจจุบันทางกลุ่มได้ clone ใส่ plasmid ไว้เพื่อใช้เป็น positive control และพัฒนาการตรวจวินิจฉัยเพื่อรองรับผู้ป่วยในอนาคต

ไวรัสไข้เลือดออกเป็นปัญหาสำคัญของประเทศและทั่วโลกโดยเฉพาะในเขตร้อน การศึกษาไวรัสไข้เลือดออกโดยเฉพาะการเกิดอาการทางตับ ทางกลุ่มให้ความสำคัญและได้ดำเนินการศึกษาเกี่ยวกับ cytokines ที่เกี่ยวข้องและขบวนการ ischemic reperfusion syndrome รวมทั้งได้ทำการศึกษาการแยกชนิด Dengue genotype โดยใช้ real time PCR

การเกิดโรคเกิดใหม่นับตั้งแต่ Nipah virus ในมาเลเซียที่มีส่วนสัมพันธ์ติดจากหมู และปี 2546 SARS ได้มีการระบาดในวงกว้างจากประเทศจีนเข้าสู่ประเทศไทย ทางกลุ่มวิจัยได้มีการรวมกลุ่มเพื่อเตรียมการศึกษาในด้านการวินิจฉัยโรคโดยใช้ศาสตร์ทางอณูชีววิทยา และหลักการในการควบคุมป้องกันการแพร่ระบาดในห้องปฏิบัติการ การศึกษาเบื้องต้นได้รับทุนสนับสนุนจากศูนย์พันธุวิศวกรรมและเทคโนโลยีแห่งชาติ สวทช. แต่เนื่องจากไม่สามารถหาตัวอย่างมาใช้ในการตรวจสอบได้ ทางกลุ่มจึงได้ทำการศึกษา coronavirus ในสุนัขและ coronavirus ที่ทำให้เกิดโรคหวัดในมนุษย์

ต้นปี 2547 ได้มีการระบาดของไข้หวัดนกอย่างกว้างขวางและเป็นปัญหาเร่งด่วนสำหรับประเทศ ทางกลุ่มวิจัยได้มีการรวมกลุ่มเกิดขึ้นระหว่างอาจารย์และนักวิจัยของคณะสัตวแพทย-ศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย และมหาวิทยาลัยมหิดล ได้ทำการศึกษาการถอดรหัสพันธุกรรมของไข้หวัดนกในไก่ทั้งตัว (ประมาณ 13 k ของ 8 segment) ผลงานดังกล่าวได้รับการตอบรับลงพิมพ์ในวารสาร Virology จากการศึกษาดังกล่าวทำให้ทราบว่าสายพันธุ์ที่มีการระบาดในประเทศไทยเป็น H5N1 และมีความสัมพันธ์ใกล้ชิดกับสายพันธุ์ A/Duck/China/319-2/03 (H5N1) ที่มีการแยกได้ทางตอนใต้ของประเทศจีนในปี 2546 โดยพบว่าในส่วน H gene ในตำแหน่ง cleavage site จะเป็น polybasic amino acid มี 20 codon deletion ใน N gene ส่วน stalk region มี 5 codon deletion ในส่วน NS และ amantadine resistant mutation หรือ polymorphism ในส่วน M2 gene

ทางกลุ่มยังได้พัฒนาการตรวจวินิจฉัยไวรัสไข้หวัดใหญ่และไวรัสไข้หวัดนกโดย Multiplex RT-PCR ในลักษณะ single step เพื่อให้ง่ายต่อการตรวจและได้ทำการ clone gene ส่วน H5, N1 และ M เพื่อเก็บไว้ใช้เป็น positive control ในการตรวจวินิจฉัยทางห้องปฏิบัติการต่อไป ผลงานได้ตอบรับเผยแพร่ในวารสาร Virial Immunology รวมทั้งร่วมมือกับห้องปฏิบัติการโรงพยาบาลรามาธิบดี (รศ.ดร.วสันต์ จันทราทิตย์) ในการพัฒนาการตรวจวินิจฉัย

นอกจากนี้ทางกลุ่มได้ร่วมมือกับคณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล ทำการถอดรหัสพันธุกรรมไวรัสไข้หวัดนกในเสือทั้ง 2 ตัว ได้ทั้งหมด 8 gene และการวินิจฉัยโดย immunohistochemistry ในส่วนเนื้อปอดของเสือโดยได้ส่ง อาจารย์จุฑาทิพย์ เขียวเจริญ ไปทำการศึกษายังประเทศเนเธอแลนด์ ผลงานดังกล่าวแสดงให้เห็นการข้าม species ของเชื้อไข้หวัดนกและ

ได้รับตอบรับลงพิมพ์เผยแพร่ในวารสาร Emerging Infectious Diseases (Impact factor 5.3) รวมทั้งยังได้ทำการศึกษาการติดเชื้อใน Avian species ต่างๆ กว่า 10 ชนิด และการศึกษาระบาดวิทยาของไข้หวัดนก อยู่ในระหว่างการดำเนินการ

ทางด้านโรคตับทางกลุ่มยังได้ทำการศึกษาโรคท่อน้ำดีตีบตับโดยเฉพาะกลไกการเกิดพังผืดและ cytokines ที่เกี่ยวข้อง

การศึกษาดังกล่าวเกิดจากความร่วมมือของกลุ่มคณาจารย์ นักวิจัย นักวิทยาศาสตร์ นิสิตปริญญาดุษฎีบัณฑิต มหาบัณฑิต จำนวนมาก ทำให้ผลงานมีความก้าวหน้ามาโดยตลอด

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1.2 บรรยายเรื่อง "การบริหารงานวิจัย" ในการประชุมโครงการสัมนาเชิงปฏิบัติการเรื่องกลยุทธ์การวิจัยสำหรับอาจารย์ใหม่ ณ ห้องประชุมสารนิเทศ หอประชุมจุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ ในวันที่ 30 ตุลาคม 2544

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1.4 บรรยายพิเศษให้ความรู้เรื่อง "ไวรัสตับอักเสบ การทำงานของนักวิชาการ นักวิจัย" และคุณค่าของงานวิจัยให้กับนักเรียนมหิดลวิทยานุสรณ์ ในการเข้าเยี่ยมชมและดูงานในศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 12 พฤศจิกายน 2544

1.5 บรรยายเรื่อง "Viral Hepatitis it treatment" บรรยายพิเศษรายวิชา PYMP 554 PYMP 554 clinical pharmacology II ของคณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล ในวันที่ 1 สิงหาคม 2545

1.6 บรรยายเรื่องปัจจุบันและอนาคตของวัคซีนและการตรวจวินิจฉัยโรคไวรัสตับอักเสบ บี (HBsAg) โดยวิธีทางพันธุวิศวกรรมเพื่อพัฒนาเป็นวัคซีนและใช้ผลิตชุดน้ำยาสำเร็จรูปตรวจหาแอนติบอดีย์ต่อเชื้อไวรัสตับอักเสบ บี (anti-HBsAg) ของสำนักงานคณะกรรมการวิจัยแห่งชาติ ณ ห้องบอลรูม โรงแรมมารวยการ์เด้นกรุงเทพฯ ในวันที่ 6 สิงหาคม 2545

1.7 บรรยายเรื่อง Viral Hepatitis ในการจัดประชุมวิชาการประจำปี คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ จังหวัดสงขลา ในวันที่ 15 สิงหาคม 2545

1.8 บรรยายเรื่อง "การบริหารโครงการวิจัยในการจัดสัมนาโครงการเส้นทางสู่นักวิจัยมืออาชีพ" (ครั้งที่ 3 ณ ห้องประชุม 401 อาคาร 3 ชั้น 4 คณะครุศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 16 สิงหาคม 2545

1.9 "New Prospect in Vaccine" ในการประชุมโครงการการสัมนาวิชาการโรคติดต่อประจำปี 2545 ของกรมควบคุมโรค ณ โรงแรมเชียงใหม่ภูคำ จังหวัดเชียงใหม่ ในวันที่ 3 กันยายน 2545

1.10 "Component of GCP+Discussion" ในการประชุม Practical GCP ฝ่ายวิจัย คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย (13.00-14.15 น) ในวันที่ 19 กันยายน 2545

1.11 "Beyond GCP + Discussion" ในการประชุมเชิงปฏิบัติเรื่อง "Practical GCP" คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 20 กันยายน 2545

1.12 กมล สกุลวิระ, อภิรดี เทียมบุญเลิศ, คณิศักดิ์ อรวีระกุล, ณรงค์ศักดิ์ ชัยบุตร, ยง ภู่วรวรรณ "ไมค์ป่วยจากการติดเชื้อ Herpes ไวรัส " ในการประชุมวิชาการประจำปีครั้งที่ 12 สมาคมไวรัสวิทยา (ประเทศไทย) ณ ห้องภาณุรังษี โรงแรมรอยัลริเวอร์ บางพลัด กรุงเทพฯ ในวันที่ 20 ธันวาคม 2545

1.13 Thanasugarn W, Samransamruajkit R, Vanapongtipgorn, P, Praphal N, Poovorawan Y. Human metapneumovirus Infection in Thai children. The Twelfth Scientific Annual. Meeting 20 December 2002, The Virology Association (Thailand)

1.14 Teeraporn C, Bruijns S, Noppornpath S, Poovorawan Y, Osterhaus A.D.M.E., Hagmans B. Detection of hepatitis C virus specific cells using epstein-bar virus based plasmids submit เพื่อพิจารณานำเสนอใน 6th FIMSA Advance Course and conference, Ayutthaya. Thailand 21-25, Oct 2002

1.15 นายสัญชัย พยุงภร นิสิตปริญญาเอกกาญจนาภิเษก หลักสูตรสาขาชีวเวชศาสตร์ คณะสหสาขา จุฬาลงกรณ์มหาวิทยาลัย ได้เข้าร่วมประชุม ในการประชุม 4th HUGO Pacific meeting and 5th Asian Pacific conference on human genetics, Education Program Genome and Health ณ โรงแรม Ambassador city Jomtien พัทยา ชลบุรี ในวันที่ 27-30 ตุลาคม 2545

1.16 "Hepatitis update" ในการบรรยายวิชาการ Basic medicial Science การฝึกอบรม 2545-2546 โรงพยาบาลชลบุรี วันที่ 26 กันยายน 2545

1.17 บรรยายเรื่อง "Hepatitis" ในการประชุมสัมนาวิชาการเรื่อง "Meet the Experts: Travelling Medicine and related vaccine" ของบริษัทเอเวนตีส ปาสเตอร์ (ประเทศไทย) จำกัด ณ โครงการพัฒนาดอยตุง พระตำหนักดอยตุง อำเภอแม่ฟ้าหลวง จังหวัดเชียงราย ในวันที่ 7-9 กุมภาพันธ์ 2546

1.18 บรรยายเรื่อง "เทคนิคการเขียนบทความ" โครงการอบรมเพื่อเพิ่มศักยภาพนิสิตบัณฑิตศึกษา บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 13 กุมภาพันธ์ 2546

1.19 บรรยายเรื่อง "ความรู้และข้อมูลใหม่ๆ เกี่ยวกับวัคซีนและยาปฏิชีวนะ" ในการประชุมเรื่อง 12th WST & A (12th Workshop on Inmunization and Antibiotics) บริษัทแกล็กโซสมิทไคล์น (ประเทศไทย) จำกัด ณ โรงแรมพาวิลเลียนควีนส์ เบย์ จังหวัดกระบี่ ในวันที่ 15-18 กุมภาพันธ์ 2546

1.20 บรรยายเรื่อง "Strategy for a successful clinical trial" ในการอบรมเรื่อง "Clinical trial : model and career" ฝ่ายวิจัย คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ณ ห้องประชุมดำรงแพทยคุณ (ห้อง 312/1) อาคาร อปร ชั้น 3 ในวันที่ 19 มีนาคม 2546

1.21 บรรยายเรื่อง "Molecular Approach to Discover New Viruese" ในการประชุมเรื่อง "Approach to Dicover New Viruses" สมาคมไวรัสวิทยา (ประเทศไทย) ร่วมกับภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ศิริราชพยาบาลและกรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข ณ ห้องประชุม NIH สถาบันวิทยาศาสตร์สาธารณสุข กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข นนทบุรี ในวันที่ 10 เมษายน 2546

1.22 Wanida Thanasugarn, Rujipat Samransamruajkit, Pijitra Vanapongtipagorn, Nuanchan Prapphal, Bernadette Van Den Hoogen Albert D.M.E Osterhaus, Yong Poovorawan. Human metapneumovirus infection in Thai children เสนอในที่ประชุมการประชุมใหญ่กุมารเวชศาสตร์แห่งประเทศไทย ครั้งที่ 56 วันที่ 23-25 เมษายน 2546

1.23 Kamol Sakulwira, Pijitra Vanapongtipagorn, Apiradee Theamboonlers, Parvapan Bhattarakosol, Siriwan Wananukul, and Yong Poovorawan Detection and Differentiation of Human Herpesviruses 1-5 by Consensus Primer PCR and RFLP^{+} เสนอในที่ประชุม การประชุมใหญ่กุมารเวชศาสตร์แห่งประเทศไทย ครั้งที่ 56 วันที่ 23-25 เมษายน 2546

1.24 A. Theamboonlers T. Chinchai, K. Bedi, P. Jantarasamee, M. Sripontong, and Y. Poovorawan. Molecular characterization of HCV core region in infected Thai blood donor เสนอในที่ประชุมการประชุมใหญ่กุมารเวชศาสตร์แห่งประเทศไทย ครั้งที่ 56 วันที่ 23-25 เมษายน 2546

1.25 Voranush Chongsrisawat, Prachya Kongtawelert, Wannarat Tongsoongnoen, Pisit Tangkijvanich, Paisarn Vejchapipat , Yong Poovorawan. Serum hyaluronan as a marker reflecting the severity of cirrhosis and portal hypertension in postoperative biliary atresia เสนอในที่ประชุม การประชุมใหญ่กุมารเวชศาสตร์แห่งประเทศไทย ครั้งที่ 56 วันที่ 23-25 เมษายน 2546

1.26 Paisarn Vejchapipat, Apiradee Theamboonlers, Rapeepan Chaokhonchai,Voranush Chongsrisawat, Soottiporn Chittmittrapap, Yong Poovorawan. Serum hepatocyte growth factor and clinical outcome in biliary atresia เสนอในที่ประชุมการประชุมใหญ่กุมารเวชศาสตร์แห่งประเทศไทย ครั้งที่ 56 วันที่ 23-25 เมษายน 2546

1.27 Yong Poovorawan, Apiradee Theamboonlers, Podchanad Jantaradsamee, Pantipa Chatchatree, Voranush Chongsrisawat Identification and characterization of clinical and subclinical hepatitis a among an outbreak in child care center. varicella infection in a pediatric aids patient presenting as umbilicated papules เสนอในที่ประชุม การประชุมใหญ่กุมารเวชศาสตร์แห่งประเทศไทย ครั้งที่ 56 วันที่ 23-25 เมษายน 2546

1.28 Theamboonlers A, Jantaradsamee P, Chatchatee P, Chongsrisawat V. Mokmula M, Poovorawan Y. Molecular characterization of hepatitis a virus infection in the context of outbreaks in the southern part of thailand เสนอในที่ประชุม การประชุมใหญ่กุมารเวชศาสตร์แห่งประเทศไทย ครั้งที่ 56 วันที่ 23-25 เมษายน 2546

1.29 บรรยายเรื่อง "ชีววิทยาและพฤติกรรมของไคโรนาไวรัสซาร์ส (Corona SARS Virus) ในการเสวนาเรื่อง "SARS... มหันตภัยที่ไทยรับมือได้" สมาคมวิทยาศาสตร์แห่งประเทศไทย ในพระบรมราชูปถัมภ์ ณ ห้องกมลทิพย์ โรงแรมสยามซิตี้ กรุงเทพฯ ในวันที่ 8 พฤษภาคม 2546

1.30 บรรยายเรื่อง "การวิเคราะห์ทางห้องปฏิบัติการสู่การประยุกต์ใช้ทางคลินิก (Laboratory Investigation : From Lab to Clinical Use) ในการประชุมวิชาการประจำปีทางเทคนิคการแพทย์ครั้งที่ 27 สมาคมเทคนิคการแพทย์แห่งประเทศไทย ณ โรงแรมแอมบาสเดอร์ซิตี้ พัทยา จังหวัดชลบุรี ในวันที่ 29 เมษายน – 2 พฤษภาคม 2546

1.31 บรรยายเรื่อง "Update in Viral Hepatitis " ในการประชุมวิชาการคณะกรรมการวิชาการ องค์การแพทย์ โรงพยาบาลหัวเฉียว ณ ห้องประชุมแพทย์ชั้น 3 โรงพยาบางหัวเฉียว ในวันที่ 12 พฤษภาคม 2546

1.32 บรรยายเรื่อง "การศึกษาทางอณูชีววิทยา" ในการสัมนาวิชาการ BIOTEC Forum เรื่อง "เทคโนโลยีชีวภาพทางการแพทย์ : SARS" ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวแห่งชาติ ณ ห้องประชุม 513 ชั้น 5 อาคารไอโอเทค อุทยานวิทยาศาสตร์ประเทศไทย คลองหลวง จังหวัดปทุมธานี ในวันที่ 28 พฤษภาคม 2546

1.33 บรรยายเรื่อง "Diagnosis of chronic hepatitis C virology Serological assays, molecular assays, HCV-genotyping" ในประชุมวิชาการเรื่อง "Immunovations of Hepatitis C Science, Clinical and Patients Management " บริษัทเชอริ่ง-พลาวจำกัด ณ ภูใจใสรีสอร์ท จังหวัดเชียงราย ในวันที่ 7 มิถุนายน 2546

1.34 บรรยายเรื่อง "SARS" อาจารย์และแพทย์ประจำบ้าน ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี ณ ห้องประชุมกุมารเวชศาสตร์ อาคาร 1 ชั้น 8 โรงพยาบาลรามาธิบดี ในวันที่ 10 มิถุนายน 2546

1.35 บรรยายเรื่อง "Viral Hepatitis และหลักการรักษา" รหัสวิชา PYPM 503 Clinical Pharmacology I คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล ณ ห้องบรรยาย 207 อาคารรัตน์ ในวันที่ 12 มิถุนายน 2546

1.36 บรรยายเรื่อง "ทำวิจัยอะไรดีในสถานการณ์ปัจจุบัน" การฝึกอบรมเรื่อง "กระบวนการวิจัยทางวิทยาศาสตร์การแพทย์และสาธารณสุขรุ่นที่ 2" สถาบันวิจัยวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ณ ห้องประชุม 221-222 ชั้น 2 อาคารสถาบัน 2 จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 23-27 มิถุนายน 2546

1.37 บรรยายเรื่อง "New Virus Discovery : SARS model" ในการเสวนาทางวิชาการ (Academic Forum) สถาบันวิจัยและพัฒนาวิทยาศาตร์และเทคโนโลยี มหาวิทยาลัยมหิดล ณ ห้องประชุมใหญ่ชั้น 1 อาคารสถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยมหิดล วิทยาเขตศาลายา จังหวัดนครปฐม ในวันที่ 27 มิถุนายน 2546

1.38 บรรยายเรื่อง "Epidemiology of Hepatitis " รายวิชา TMHG 501: Tropical Hygiene หลักสูตรนานาชาติ คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล ณ ห้องบรรยายชั้น 3 ตึกจำลองหะริณสุต ในวันที่ 30 มิถุนายน 2546

1.39 บรรยายเรื่อง "การค้นพบไวรัส SARS และไวรัสตัวใหม่" ในการอบรมเชิงปฏิบัติการเรื่อง "มาตรการป้องกันและการตรวจวินิจฉัยโรค SARS ทางห้องปฏิบัติการ" สมาคมเทคนิคการแพทย์แห่งประเทศไทย ณ โรงแรมเมอร์ชั่นคอร์ท กรุงเทพฯ ในวันที่ 2 กรกฎาคม 2546

1.40 บรรยายเรื่อง "How to Develop Successful Research" อบรมหลักสูตรการทำวิจัย กลุ่มวิจัยและพัฒนา สถาบันสุขภาพเด็กแห่งชาติมหาราชินี ณ ห้องประชุมจักรพันธุ์ โปษยกฤต ชั้น 7 อาคารสยามบรมราชกุมารี ในวันที่ 3 กรกฎาคม 2546

1.41 บรรยายเรื่อง "SARS Discovery" ประชุมวิชาการครั้งที่ 5 ประจำปี 2546 ศูนย์บริการโลหิตแห่งชาติ สภากาชาดไทย ณ ห้องประชุมศิริ สิริโยธินชั้น 4 ในวันที่ 11 กรกฎาคม 2546

1.42 บรรยายเรื่อง "Viral hepatitis" การเรียนการสอนรวมภาควิชา สำหรับนักศึกษาแพทย์ชั้นปีที่ 4, 5, Extern แพทย์ประจำบ้าน อาจารย์และบุคลากรทางการแพทย์ คณะแพทยศาตร์ โรงพยาบาล รามาธิบดี มหาวิทยาลัยมหิดล ณ ห้องประชุมอารีวัลยะเสวี วันที่ 18 กรกฎาคม 2546

1.43 บรรยายเรื่อง "Values mandate" ฝึกอบรมหลักสูตร การพัฒนาการบริหารสำหรับผู้บริหาร คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย รุ่นที่ 2 ณ ห้อง 605/1 ชั้น 6 ตึก อปร. คณะแพทย-ศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 30 กรกฎาคม 2546

1.44 บรรยายเรื่อง "ปัญหาและการป้องกันไวรัสตับอักเสบในประเทศไทย" ณ ห้อง 202 อาคารจามจุรี 4 จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 29 สิงหาคม 2546

1.45 บรรยายเรื่อง " Clinial trial : publication & academic promotion" ห้องประชุมทวี ตุมราศวิน (ห้อง M10/1-3) อาคาร อปร. ชั้น M ในวันที่ 20 สิงหาคม 2546

1.46 บรรยายเรื่อง "การวินิจฉัยโรคไวรัสทางห้องปฏิบัติการ (Molecular Diagnosis)" ห้องประชุมสดศรี วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้าฯ ในวันที่ 16 กันยายน 2546

1.47 บรรยายเรื่อง "โรคติดต่ออุบัติการใหม่และอุบัติซ้ำ" ณ โรงแรม ลี การ์เดนส์ พลาซ่า อำเภอหาดใหญ่ จังหวัดสงขลา ในวันที่ 18-20 สิงหาคม 2546

1.48 บรรยายเรื่อง "การบริหารจัดการหน่วยวิจัยและงบประมาณ" สำนักบริหารวิชาการ อาคารจามจุรี 5 ชั้น 6 จุฬาลงกรณ์มหาวิทยาลัย วันที่ 18 กรกฎาคม 2546

1.49 บรรยายเรื่อง "บทบาทงานวิจัยและการใช้ผลการวิจัยทางการแพทย์เพื่อพัฒนาสาธารณสุข " ห้องประชุมอาคาร อปร ชั้น 19 จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 13 ตุลาคม 2546

1.50 บรรยายเรื่อง "ความก้าวหน้าการวินิจฉัยอณูชีวของไวรัสตับอักเสบในประเทศไทย" ณ ห้อง M 10/3 ตึก อปร. จุฬาลงกรณ์มหาวิทยาลัย วันที่ 15 ตุลาคม 2546

1.51 บรรยายเรื่อง "ไวรัสตับอักเสบและการป้องกัน" ณ โรงแรมนารายณ์ กรุงเทพฯ ในวันที่ 31 ตุลาคม 2546

1.52 บรรยายเรื่อง "การวินิจฉัยโรคไวรัสทางห้องปฏิบัติการ Molecular Diagnosis และหลักการ Real-time PCR" ณ ห้องประชุม คณะวิทยาศาสตร์การแพทย์ มหาวิทยาลัยนเรศวร พิษณุโลก ในวันที่ 17 พฤศจิกายน 2546

1.53 บรรยายเรื่อง " Recent advances in viral diagnosis" The 5th Colloquium of Asian Network for Clinical Laboratory Standardization and Harmonization (ANCLS) Advanced Technology in Laboratory Medicine 17-19 December 2003

1.54 บรรยายเรื่อง "นักวิจัยรุ่นใหม่พบเมธีวิจัยอาวุโส สกว." ณ โรงแรมเฟลิกว์ ริเวอร์แคว จังหวัดกาญจนบุรี ในวันที่ 9-11 มกราคม 2547

1.55 บรรยายเรื่อง "Hepatitis Vaccine" หน่วยโรคติดเชื้อ ภาควิชาอายุรศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 22 มกราคม 2547

1.56 บรรยายเรื่อง "Authorship" ณ ห้องประชุมดำรงแพทยาคุณ ชั้น 3 ตึก อปร. จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 28 มกราคม 2547

1.57 บรรยายเรื่อง "โรคตับอักเสบจากเชื้อไวรัส" ณ ห้องประชุม 1-301 อาคารอาทิตย์ อุไรรัตน์ มหาวิทยาลัยรังสิต ในวันที่ 30 มกราคม 2547

1.58 บรรยายเรื่อง "ไวรัสตับอักเสบ บี กับบุคลากรทางการแพทย์" ณ ห้องประชุมศาสตราตราจารย์ประสพ รัตนากร ชั้น 3 อาคารอำนวยการ สถาบันประสิทวิทยา กรุงเทพฯ ในวันที่ 9 กุมภาพันธ์ 2547

1.59 บรรยายเรื่อง "วิกฤติไข้หวัดนก บทเรียนสำหรับอนาคต" ห้อง 105 อาคารมหาจุฬาลงกรณ์ จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 23 กุมภาพันธ์ 2547

1.60 บรรยายเรื่อง "Molecular Epidemiology of Flu and Its Application และ Specimen and Data collection Transportation, Safety Practice" ณ ห้องประชุม 1 ตึกอำนวยการ ชั้น 6 สถาบันบำราศนราดูร ในวันที่ 26 กุมภาพันธ์ 2547

1.61 บรรยายเรื่อง "Liver diseases in infants & children" ณ อาคารเฉลิมพระบารมี 50 ปี ชั้น P.3 ซอยศูนย์วิจัย ถนนเพชรบุรี กรุงเทพฯ วันที่ 27 กุมภาพันธ์ 2547

1.62 บรรยายเรื่อง "Viral subtype : HIV, HBV, HCV" ณ ห้องควีนส์ปาร์ค 1-2 โรงแรมอิมพีเรียล ควีนส์ปาร์ค กรุงเทพฯ วันที่ 16 มีนาคม 2547

1.63 บรรยายเรื่อง "ผลของการกลายพันธ์ในส่วน 'a' determinant ของ HBsAg ต่อชุดตรวจคัดกรอง" ณ โรงแรมอิมพีเรียล ควีนส์ปาร์ค กรุงเทพฯ วันที่ 15 มีนาคม 2547

1.64 บรรยายเรื่อง "Problem of viral hepatitis and clinical application in Asia" ณ ห้องประชุม Monet ชั้น 4 โรงแรมโนโวเทล สยามสแควร์ กรุงเทพฯ ในวันที่ 1 เมษายน 2547

1.65 บรรยายเรื่อง "ไข้หวัดนก" ณ เวทีใหม่ สวนอัมพร กรุงเทพฯ ในวันที่ 5 เมษายน 2547

1.66 บรรยายเรื่อง"Avian flu : How scared should you be? H5N1 influenza virus in Thailand" ณ ห้อง Grand Ballroom โรงแรมเอดิสัน ถนนพระราม 9 กรุงเทพฯ ในวันที่ 29 เมษายน 2547

1.67 บรรยายเรื่อง "กลยุทธ์ในการทำวิจัยทางคลินิก" ณ ห้องประชุมนายแพทย์สุจินต์ ผลากรกุล โรงพยาบาลชลบุรี ในวันที่ 10 พฤษภาคม 2547

1.68 บรรยายเรื่อง "ผลการถอดรหัสพันธุกรรมไข้หวัดนก" ณ ห้องสาธิต ตึก 60 ปี คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 20 พฤษภาคม 2547

1.69 บรรยายเรื่อง "งานวิจัยกับการเป็นผู้นำของสังคม" ณ ห้องเฉลิม พรมมาส ตึก อปร. จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 28 พฤษภาคม 2547

1.70 บรรยายเรื่อง "Biliary atresia and Organ Transplantation" ณ ห้องประชุม ศ.ทพ.ประพันธ์ พืชผล ภาควิชาทันตกรรมสำหรับเด็ก ตึกทันตรักษ์วิจัย คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ในวันที่ 29 มิถุนายน 2547

1.71 บรรยายเรื่อง "Viral hepatitis" ณ ห้องบรรยายชั้น 3 ตึกจำลอง หริณสุต คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล ในวันที่ 20 กรกฎาคม 2547

2. ผลงานที่ลงพิมพ์ในวารสารระดับนานาชาติ ข้อมูล รวมระยะเวลา 3 ปี ผลงานวิจัยที่ได้รับการเผยแพร่หรือตอบรับการเผยแพร่

No.	ได้มีการเผยแพร่หรือ accepted	วารสาร	impact factor
1	Vorasuk Shotelersuk, Chupong Ittiwut, Sumarlee Srivuthana, Suthipong acharasindhu, Suphab Aroonparkmongkol, Apiwat Mutirangura, Yong Poovorawan Clinical and Molecular Characteristics of Thai Patients with Achondroplasia	Southeast Asian J Trop Med and Public Health 2001;32:429-33.	-
2	Ilina Isahak, On behalf of the Steering Committee for Prevention and Control of Infectious Diusease in Asia. Adult immunization A neglected issue in southeast asia	Southeast Asian J Trop Med Pub Health 2000;31:173-184.4	-

3	Pisit Tangkijvanich, Duangporn Thong-ngam, Varocha Mahachai, Nusont Kladchareon, Pongspeera Suwangool Pinit Kullavanijaya Long-term Effect of Interferon Therapy on Incidence of Cirrhosis and Hepatocellular Carcinoma in Thai Patients with Chronic Hepatitis B	Southeast Asian J Trop Med Pub Health 2001;32:452-8	--
4	Theamboonlers A, Hansurabhanon T, Verachai V, Chongsrisawat V, Poovorawan Y Hepatitis D virus infection in Thailand: HDV genotyping by RT-PCR, RFLP and direct sequencing	Infection 2002;30:140-4.	1.04
5	Likitnukul S, Chatchatee P, Chongsrisawat V, Poovorawan Y. Typhoid fever :	Vaccine children & Practice 2001;4:34-38	-
6	Poovorawan Y, Chongsrisawat V, Tangkijvanich P. Problems and prevention of Viral Hepatitis in Thailand	J Med Assoc Thai 2001;84suppl:518-525	-
7	Poovorawan Y, Chatchatee P, Chongsrisawat V. Hepatitis B vaccine failure.	Indian Journal of Gastroenterology. 2000;19:p20 (Abstract)	-
8	Kongruttanachok N, Sukdikul S, Setavarin S, Kerekhjanarong V, Supiyaphun P, Voravud N, Poovorawan Y, Mutirangura A. Cytochrome P450 2E1 polymorphism and nasopharyngeal carcinoma development in Thailand: a correlative study	BMC Cancer 2001 1-4. http://www.biomedcentral.com/1471-2407/1/4	
9	Vorasuk Shotelersuk, Chupong Ittiwut, Kanjana Shotelersuk, Surang triratanachat, Yong Poovorawan, Apiwat Mutirangura Fibroblast Growth Factor Receptor 3 S249C Mutation in Viral Associated Squamous Cell Carcinomas.	Oncology report 20001;8:1301-4.	1.25
10	Lolekha S, Cooksley G, Chan V, Isahak I, Ismael S, John J, Khiem HB, Kunasol P, Wah LB, Seong NH, Paje-Villar E, Sulaiman HA, Poovorawan Y, Steering committee for prevention and control of infection disease in Asia. A review of Hib epidemiology in Asia.	Southeast Asian J Trop Med Public Health 2000;31:650-7.	-

11	Yong Poovorawan, Pantipa Chatchatee, Voranush Chongsrisawat, Epidemiology and prophylaxis of viral hepatitis - a global perspective.	J Gastroenterol Hepatol 2002;17:S156-167	1.53
12	Hansurabhanon T, Jirapongsa C, Tunsakun P, Sukbunsung R, Bunyamanee B, Kuirat P, Meedsen S, Waedeng W, Theamboonlers A, Poovorawan Y. Infection with hepatitis C virus among intravenous-drug users: prevalence, genotypes and risk-factor-associated behaviour patterns in Thailand.	Ann.Trop. Med. Parasitol. 2002;96:615-625.	1.04
13	Viroj Verachai, Tipwan Rhutiprawan, Apiradee Theamboonlers, Teeraporn Chinchai, Srivilai Tanprasert, Bart Haggman A.D.M.E. Osterhaus, Yong Poovorawan. The prevalence and genotypes of hepatitis C virus infection among different groups of drug addicts and blood donors.	8th International Symposium on Hepatitis C & Related virused : Molceular Viology and Pathogenesis : Sep 2-5,2001 Paris, France 2001 (abstract)	-
14	Chongsrisawat V, Poovorawan T. Management of chronic hepatitis B & C virus infections.	Indian J Pediatr 2002;16:149-154	-
15	Pantipa Chatchatee, Voranush Chongsrisawat, Apiradee Theamboonlers, Yong Poovorawan. Declining Hepatitis A Seroprevalence Among Medical Students in Bangkok,Thailand, 1981-2001.	Asian Pacific Journal Allergy Immunol 2002;20:53-56.	0.27
16	Kamol Sakulwira, Phingphol Charoonrut, Parntep Ratanakorn. Yong Poovorawan Serological Evidence of Herpesvirus Infections in Gibbons.	BMC Microbiol. 2002 May 31;2(1):11.	-
17	Chongsrisawat V, Chatchatee P, Samransamruajkit R, Vanapongtipagorn P, Chottivittayatarakorn P, Poovorawan Y. Plasma endothelin-1 levels in patients with biliary atresia: possible role in development of portal hypertension.	Ped Surg Int 2003;6:478-81.	1.44
18	Viroj Verachai, Tipwan Thutiprawan, Apiradee Theamboonlers, Teeraporn Chinchai, Srivilai Tanprasert, Bart L. Haagmans, Albert.-D.M.E. Osterhaus, Yong Poovorawan.Prevalence and Genotypes Of Hepatitis C virus infection among different groups of drug addicts and blood donors in Thailand	Southeast Asian J Trop Med Public Health 2002;4:849-851.	-

19	Kosuwon P, Bunyarat W, Baugerd T, Hutagalung Y, Bock HL, Poovorawan Y, Reduced antigen content diphtheria-tetanus-acellular pertussis vaccine (dTpa) as a booster dose for 4 to 6 year-old children immunized with 4 doses of whole cell pertussis vaccine	Vaccine. 2003;21:4194-4200	3.00
20	Kamol Sakulwira, Pijitra Vanapongtipagorn, Kanisak Oraveerakul, Yong Poovorawan Prevalence of Canine Coronavirus and Parvovirus Infections in Dogs with Gastroenteritis.	Veterinary Medicine 2003;48:163-167	0.60
21	Rujipat Samransamruajkit, Kittichai Moonviriyakit, Pijitra Vanapongtipagorn, Nuanchan Prapphal.Jitladda Deelodegenavong, Yong Poovorawan Y. Plasma endothelin-1 in infants and young children with acute bronchiolitis and viral pneumonia.	Asian Pac J Allerg Immunol 2002;20:229-234.	0.27
22	Klanrit P, Thongprasom K, Rojanawatsirivej S, Theamboonlers A, Poovorawan Y. Hepatitis C virus infection in patients with oral lichen planus.	Oral Diseases. 2003;9:292-297	1.01
23	Pisit Tangkijvanich, Apiradee Theamboonlers, Maturapod Sriponthong, Yong Poovorawa. SEN Virus infection in patients with chronic liver disease and hepatocellular carcinoma.	J Gastroenterol 2003;38:142-148	1.17
24	Vorayod Boonyarad, Sommai Choeichareon, Anuchit Chutaputti, Yong Poovorawan.Interspousal Transmission of Hepatitis C virus in thailand	J. Gastroenterol 2003;38:1053-1059	1.19
25	Suwanna Noppornpanth, Bart L. Haagmans, Parvapan Bhattarakosol, Parntep Ratanakorn, Albert D.M.E. Osterhaus and Yong Poovorawan. Molecular epidemiology of gibbon hepatitis B virus transmission	J Gen Virol. 2003;84:147-155	3.24
26	Siriwan Wananukul, Vanida Nopponpunth, Yong Poovorawan. Human herpesviruses infection in children with fever and maculopapular rash	Asian Pacific J Allerg Immunol 2004 ;21:217-21	1.04

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32	Sanipa Suradhat, Roongroje Thanawongnuwech, Yong Poovorawan. Up regulation of IL-10 gene expression in porcine peripheral blood mononuclear cells by porcine reproductive and respiratory syndrome virus (PRRSV).	J Gen Virol 2003;84:453-459.	3.03
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35	Jutamas Umpuchineewan, Siriwan Wananukul, Kamol Sakulwira, Yong Poovorawan. Varicella Infection in Pediatric AIDS Patient Presenting as Umbilicated Papules	Asian Pac J Allergy Immunol. 2003 Mar;21 (1):63-8.	0.27
36	Tangkijvanich P, Theamboonlers A, Sriponthong M, Kullavanijaya P, Poovorawan Y. SEN Virus infection and the risk of hepatocellular carcinoma: A Case control study	Am J Gastroenterol 2003;9:292-297	4.17
37	Paisarn Vejchapipat, Apiradee Theambonlers, Rapeepan Chaokhonchai, Voranush Congsrisawat , Soottiporn Chittmittrapap, Yong Poovorawan. Serum hepatocyte growth factor and clinical outcome in biliary atresia	J Pediatr Surg. 2004;39:1045-1049.	1.44
38	Voranush Chongsrisawat, Prachya Kongtawelert, Wannarat Tongsoongnoen, Pisit Tangkijvanich, Paisarn Vejchapipat, Yong Poovorawan. Serum hyaluronan as a marker reflecting the severity of cirrhosis and Portal hypertension in patients with postoperative biliary atresia	Ped Surg Int 2003 (inpress)	1.44
39	Sasithorn Likitnukul, Parvapan Bhattarakosol, Yong Poovorawan Seroprevalence of cytomegalovirus infection in children born to hiv-1 infected women	Asian Pac J Allerg Immunol 2003;21:127-30.	0.27
40	Prakat Charearnrad,Voranush Chongsrisawat, Supatporn Tepmongkol,Yong Poovorawan. Effect of Phenobarbital on The Accuracy of ^{99m}Tc-DISIDA Scintigraphy in Differentiation Biliary Atresia from Neonatal Hepatitis Syndrome	J Med Assoc Thailand 2003;86:S189-S198	-
41	Kamol Sakulwira, Apiradee Theamboonlers, Kanisak Oraveerakul, Narongsak Chaiyabutr, Parvapan Bhattarakosol, and Yong Poovorawan Orangutan lymphocryptovirus	J Med Primatology 2004;33:25-9.	0.78

42	Pisit Tangkijvanich, P Kongtawelert, Varocha Mahachai, Pongspeera Suwangool and Yong Poovorawan. Serum Hyaluronan: A Marker of Liver Fibrosis in Patients with Chronic Liver Disease	Asian Pac J Allerg Immunol 2003;21:115-20.	0.27
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45	Chirathaworn C, Pongpanich A, Poovorawan Y. Herpes Simplex virus 1 induced LOX-1 Expression in an Endothelial Cell Line, ECV 304	Viral Immunol 2004;17:307-314	1.94
46	Nuchprayoon S, Porksakorn c, Junpee A, Sanprasert V, Poovorawan Y. Comparative assessment of an Og4C3 ELISA and an ICT Filariasis Test : A study of Myanmar migrants in Thailand.	Asian Pac J Allergy and Immunol. 2003;21:253-257	0.27
47	Poovorawan Y, Chongsrisawat V. Definition and Epidemiology of Acute Liver Failure. Proceeding of the 2nd World Congress of Pediatric Gastroenterology.	Hepatology and Nutrition Palais des congres Paris . 2004;3-7 July 2004. (proceding)	
48	Nuchprayoon S, Sangprakarn S, Jundee A, Nithiuthai S, Chungpivat S, Poovorawan Y. Differentiation of Brugia malayi and Brugia pahongi by PCR-RFLP of ITS-1 and ITS 2.	Southeast Asian J Trop Med Pub Health 2003;34:67-73	-
49	Sasithorn Likitnukul, Nuanchan Prapphal, Wiroje Pongpunlert, Pornchai Kingwatanakul, Yong Poovorawan. Dual infection : Dengue hemorrhagic fever with unusual manifestations and mycoplasma pneumonia in a child	Southeast Asian J Trop Med Pub Health 2004 (inpress)	-

50	Yong Poovorawan, Apiradee Theamboonlers, Voranush Chongsrisawat, Pojchanad Jantaradsamee, Soontaree Chutsirimongkol, Pisit Tangkijvanich, Clinical features and molecular characterization of hepatitis A virus outbreak in a child care center in Thailand	J Clinical Virol 2004 (accepted)	2.02
51	Voranush Chongsrisawat, Apiradee Theamboonlers, Yong Poovorawan. Prevalence of hepatitis A virus infection in children with biliary atresia.	Southeast Asian J Trop Med 2003 (accepted)	-
52	Paisarn Vejchapipat, Pisit Tangkijvanich, Apiradee Theamboonlers Voranush Chongsrisawat, Soottiporn Chittmittrapap, Yong Poovorawan. Association between serum hepatocyte growth factor and the survival in untreated hepatocellular carcinoma.	J Gastroenterol 2004 (accepted)	1.17
53	Sunchai Payungporn, Pisit Tangkijvanich, Pojchanad Jantaradsamee, Apiradee Theamboonlers, Yong Poovorawan, Simultaneous Quantification and Genotyping of Hepatitis B Virus by Real-Time PCR and Melting Curve Analysis	J Virol Method 2004;120:131-140	1.82
54	Suda Louisirirotchanakul, Chinda Kanoksinsombat, Apiradee Theamboonlert, Pilaipan Puthavatana, Chantapong Wasi, Yong Poovorawan. Mutation of 'a' Determinant of HBsAg with Discordant HBsAg Diagnostic Kits.	Viral Immunol (accepted) 2004	1.94
55	Nareerat Viseshakul, Roongroje Thanawongnuwech, Alongkorn Amonsin, Sanipa Suradhat, Kanisak Oraveerakul, Piya Wongyanin, Juthatip Keawchareon, Sukanya Plitkul, Apiradee Theamboonlere, Sunchai Payungporn and Yong Poovorawan. The Genome Sequence Analysis of H5N1 Avian Influenza A Virus Isolated from the Outbreak Among Poultry Populations in Thailand	Virology. 2004 (accepted)	3.39
56	Poovorawan Y. Perspectives on hepatitis B prevention through vaccination.	Vaccines: Children and Practice 2004 (inpress)	-

3. ผลงานวิจัยที่อยู่ระหว่างการพิจารณาจากวารสาร

No.	ผลงานวิจัยที่อยู่ระหว่างการพิจารณาจากวารสาร	วารสาร	Impact factor
1	Teeraporn Chinchai, Bart L. Haagmans, Sven Bruijns, Chintana Chirathaworn, Kavita Bedi, Apiradee Theamboonlers, Albert D.M.E. Osterhaus, and Yong Poovorawan . T Cell Response to Hepatitis C Virus Southeast Asian Blood Donors.	Vial immunology 2004 (submitted)	1.94
2	Rujipat Samransamruajkit, Nuanchan Prapphal, Jitladda Deelodegenavong, Yong Poovorawan, Soluble Intercellular Adhesion Molecule-1 (sICAM-1) in Pediatric ARDS during high frequency oscillatory ventilation: A randomized controlled trial, A predicted of Mortality.	2003 J Crit Care (revised)	1.29
3	Zahid Hussain, Syed Akhtar Husain, Abdul Malik, Mohammad Asim, Yong Poovorawan, Apiradee Theamboonlers, Premashish Kar. Clinical and molecular characterization of Hepatitis A virus isolates from Northern India.	J Gastro Hepatol 2003 (submitted)	1.53
4	J Keawcharoen, A Theamboonlers, P. Jantaradsamee, A Rungsipipat, K Oraveerakul. Y Poovorawan, Nucleotide sequence analysis of nucleocapsid protein gene of canine distemper virus isolates in Thailand	Vet Microbiol 2004.(revised)	1.57
5	Paisarn Vejchapipat, Naruemol Jirapanakorn, Apiradee Theamboonlers, Voranush Chongsrisawat, Soottiporn Chittmittrapap, Yong Poovorawan. K469E ICAM-1 Gene Polymorphism and Serum ICAM-1 Levels in Biliary Atresia	J Pediatr Surg 2004 (submitted)	1.44
6	Sittisak Honsawek, Voranush Chongsrisawat, Paisarn Vejchapipat, Pisit Tangkijvanich, Yong Poovorawan. Serum Interleukin-8 in Children with Biliary Atresia.	Pediatr Surg Int 2004 (revised)	1.44

7	Vimolket T, Poovorawan Y An Economic Evaluation Of Universal Infant Vaccination Strategies Against Hepatitis B In Thailand: An Analytic Decision Approach To Cost Effectiveness.	Southeast Asian J Trop Med Pub Health 2004 (submitted)	-
8	Juthatip Keawcharoen, Kanisak Oraveerakul, Thijs Kuiken†, Ron A.M. Fouchier, Alongkorn Amonsin, Sunchai Payungporn, Suwanna Noppornpanth, Sumitra Wattanodorn, Apiradee Theamboonlers, Rachod Tantilertcharoen, Rattapan Pattanarangsan, Nlin Arya, Pantep Rattanakorn, Albert D.M.E.Osterhaus, Yong Poovorawan‡Avian influenza A (H5N1) virus fatal for tigers and leopards	Emerging Infectious Disease 2004 (Submitted)	5.34
9	Sunchai Payungporn, Piraya Phakdeewirot, Salin Chutinimitkul Apiradee Theamboonlers, Juthatip Keawcharoen, Kanisak Oraveerakul, Yong Poovorawan Single Step Multiplex Reverse Transcription-Polymerase Chain Reaction (RT-PCR) for Influenza A Virus Subtype H5N1 Detection.	Viral Immunology 2004 (submitted)	1.94
10	A Theamboonlers, M. Veravigrom O, Yambangyang, P. Trairatvorakul, V. Chongsrisawat, and Y. oovorawan. The changing trends of rotavirus genotypes in Thailand	Acta Virologica 2004 (submitted)	0.68

4. ผลงานวิจัยที่ลงพิมพ์วารสารในประเทศ

1. นฤมล จิรพนากร ยง ภู่วรวรรณ Severe Acute Respiratory Syndrome คลินิก 2546;19:พิเศษ 1-12
2. ยง ภู่วรวรรณ จุดกำเนิด SARS คลินิก 2546;19:514-516
3. กิตติยศ ภู่วรวรรณ, ยง ภู่วรวรรณ ฝีดาษลิง (ในคน) คลินิก 2546;19:571-574

4. ยง ภู่วรวรรณ พรรณทิพา ฉัตรชาตรี วัคซีนรวมในเวชปฏิบัติ คลินิก 2546;190-194
5. กิตติยศ ภู่วรวรรณ, ยง ภู่วรวรรณ Human Parvovirus B19 คลินิก 2547;100-104
6. ยง ภู่วรวรรณ การให้วัคซีนสำหรบผู้เดินทางต่างประเทศหรือผู้ท่องเที่ยว 2547;1040-1043
7. วนิดา ธนสุกาญจน์, ยง ภู่วรวรณ ปอดอักเสบ Humen metapneumovirus โรคเก่าที่พบใหม่ 2547;1-4
8. ยง ภู่วรวรรณ การถอดรหัสพันธุกรรมไวรัสไข้หวัดนก คลินิก 2547;201-204
9. ยง ภู่วรวรรณ ภัทรธิดา สงวนหมู่ ไข้หวัดใหญ่และไข้หวัดนก 2047;91-99
10. ยง ภู่วรวรรณ วิธีการให้วัคซีนในเวชปฏิบัติ 2547;190-1974

5. เป็น reviewer บทความทางวิชาการในวารสารต่าง ๆ

- Vaccine
- J Gastro Hepatol
- Asian Pac J Aller Immunol
- J Med Assoc Thailand
- Int Pediatr
- Vaccine Children & practice
- Drug
- etc.

6. เป็นกองบรรณาธิการวารสาร

- J Pediatric
- ScienceAsia
- J Med. Assoc. Thai
- Vaccine Children & practice

- Chula Med. J
- J Thai Pediatric
- J Thai Pediatrics
- etc

7. ผลงานที่ได้รับรางวัล

1. รางวัลมหาวิทยาลัยมหิดล-บี บราวน์ เพื่อการแพทย์และสาธารณสุขไทย ประจำปี 2546 เรื่อง "ปัญหาและการป้องกันไวรัสตับอักเสบในประเทศไทย" กองบริหารงานวิจัยมหาวิทยาลัยมหิดล
2. รางวัลผลงานการได้รับการอ้างอิงสูงสุดจาก สกว. พ.ศ. 2546

8. รายชื่อนิสิต

นิสิตปริญญาเอก หลักสูตรจุลชีววิทยาทางการแพทย์

(จบการศึกษาแล้ว 2 คน)

นางสาวสุวรรณา นพพรพันธุ์ จบการศึกษาแล้ว

อาจารย์ธีรพร ชินชัย จบการศึกษาแล้ว

อ.นสพ.กมล สกุลวิระ ต้องออกจากการศึกษาเพราะสอบไม่ผ่าน qualify.

นิสิตปริญญาเอก หลักสูตร ชีวเวชศาสตร์

กำลังศึกษาอยู่ 3 คน

นายสัญชัย พยุงภร กำลังศึกษาอยู่

นางสาวสลิล ชุตินิมิตรกุล กำลังศึกษาอยู่

นายทวีศักดิ์ เชี่ยวชาญศิลป์ กำลังศึกษาอยู่

นิสิตปริญญาโท หลักสูตรอณูชีววิทยาทางการแพทย์

จบการศึกษา 4 คน กำลังศึกษาอยู่ 4 คน

นางสาวทรงพรรณ แสงประกาย จบการศึกษาแล้ว

นางสาวปฐมวดี เนตรมุกดา จบการศึกษาแล้ว

นางสาวพจชนาถ จันทรรัศมี จบการศึกษาแล้ว
นางสาววนิดา ธนสุกาญจน์ จบการศึกษาแล้ว
นางสาวปิรยา ภักดีวิโรจน์ กำลังศึกษาอยู่
นางสาวภัทรธิดา สงวนหมู่ กำลังศึกษาอยู่
นายกมล สุวรรณการ กำลังศึกษาอยู่
นางสาวกนกกาญจน์ บารมีชัย กำลังศึกษาอยู่

นิสิตปริญญาโท หลักสูตรสหสาขา จุลชีววิทยา
กำลังศึกษาอยู่ 2 คน
นางสาวศิริวรรณ ฐิติพงศ์ประภัทร กำลังศึกษาอยู่
สพ.ญ. อัญญารัตน์ ต้นธีรวงศ์ กำลังศึกษาอยู่

นิสิตปริญญาโท หลักสูตรชีวเคมีทางการแพทย์
กำลังศึกษาอยู่ 1 คน
นางสาววีราภา จันทรสุพิศ

นิสิตปริญญาโท กุมารเวชศาสตร์
จบการศึกษาแล้ว 4 คน
นายแพทย์กิตติชัย มูลวิริยกิจ จบการศึกษาแล้ว
แพทย์หญิงมณฑิตา วีรวิกรม จบการศึกษาแล้ว
แพทย์หญิงจัญจุรี สมาธิ จบการศึกษาแล้ว
แพทย์หญิงศิรินุช อำไพ จบการศึกษาแล้ว

<u>ที่ปรึกษาร่วม</u>
นิสิตปริญญาโท คณะเศรษฐศาสตร์
จบการศึกษาแล้ว 1 คน
1. นางสาวศุภรัตน์ เรณุมาศ จบการศึกษาแล้ว

นิสิตปริญญาโท คณะสัตวแพทยศาสตร์

จบการศึกษาแล้ว 2 คน

1. นางสาวจุฑาทิพย์ เขียวเจริญ จบการศึกษาแล้ว
2. นางสาวนุสรา พันธ์ประภา จบการศึกษาแล้ว

นิสิตปริญญาโท จุลชีววิทยา คณะแพทยศาสตร์ ศิริราชพยาบาล มหาวิทยาลัยมหิดล (ที่ปรึกษาร่วม) จบการศึกษาแล้ว 2 คน

1. นางสาวสุจิตรา แก้วระวัง จบการศึกษาแล้ว
2. นางสาวปิยะพร รัตนนิลเรือง จบการศึกษาแล้ว

สรุปผลการศึกษาของนิสิต

นิสิตดุษฎีบัณฑิต

จบการศึกษาแล้ว	2	คน
กำลังศึกษาอยู่	3	คน

นิสิตมหาบัณฑิต

จบการศึกษาแล้ว	13	คน
กำลังศึกษาอยู่	7	คน

รายชื่อนิสิตฝึกงาน

ภาควิชาชีวเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

วันที่ 1 เมษายน – 30 เมษายน 2545

นางสาวฐานิดา วุฒิกุลวาณิช	นิสิตคณะวิทยาศาสตร์
นางสาวปุณยนุช ตรีเพชราภรณ์	นิสิตคณะวิทยาศาสตร์
นางสาวสิรินทร์ สุจินตะบัณฑิต	นิสิตคณะวิทยาศาสตร์
นางสาวจิตติมา โชติวรานนท์	นิสิตคณะวิทยาศาสตร์
นางสาวภัรธิดา สงวนหมู่	นิสิตคณะวิทยาศาสตร์
นายสมลักษณ์ ลีลากิจไพศาล	นิสิตคณะวิทยาศาสตร์
นายณัฐพงษ์ เลิศเยาวฤทธิ์	นิสิตคณะวิทยาศาสตร์

วันที่ 16 เมษายน-17 พฤษภาคม 2545

นางสาวนนทิชา แจ่มกังวาล นิสิตคณะวิทยาศาสตร์

นายรัฐพล เฉลิมโรจน์ นิสิตคณะวิทยาศาสตร์

นางสาวจอมขวัญ มีรักษ์ นิสิตคณะวิทยาศาสตร์

วันที่ 10-28 มีนาคม 2546

นายมนัสสิทธิ์ บุญคงชู นิสิตคณะวิทยาศาสตร์

วันที่ 10 มีนาคม – 11 เมษายน 2546

นายกมล สุวรรณการ นิสิตคณะวิทยาศาสตร์

วันที่ 1 เมษายน – 30 เมษายน 2546

นางสาวมยุรา ทองช่วง นิสิตคณะวิทยาศาสตร์

นางสาวฐานิศร์ สมรรถวิทยาเวช นิสิตคณะวิทยาศาสตร์

วันที่ 21 เมษายน – 23 พฤษภาคม 46

นางสาวปิติรัตน์ บุญสุข นิสิตคณะวิทยาศาสตร์

นายบรรจง เตือนวีรเดช นิสิตคณะวิทยาศาสตร์

วันที่ 1 พฤษภาคม – 30 พฤษภาคม 2546

นายกิตติพงษ์ วังทะพันธ์ นิสิตคณะวิทยาศาสตร์

นางสาวณัฐณิชา พรประสิทธิ์ นิสิตคณะวิทยาศาสตร์

วันที่ 1-30 พฤษภาคม 2547

นางสาวจิตวดี พิทักษ์โรจน์กุล นิสิตคณะวิทยาศาสตร์

นางสาวขวัญชนก ศรัทธาสุข นิสิตคณะวิทยาศาสตร์

วันที่ 1-30 เมษายน 2547

นางสาวอัญชลี เกียรติวุฒินนท์ นิสิตคณะวิทยาศาสตร์

นายสาวพันธุ์ทิพา สนธิพันธ์ นิสิตคณะวิทยาศาสตร์

วันที่ 19เมษายน – 28 พฤษภาคม 2547

นายวัชรินทร์ เพชรมณีนิลใส นิสิตคณะวิทยาศาสตร์

นายจักรชัย วรรณศรี นิสิตคณะวิทยาศาสตร์

วันที่ 10 -22 พฤษภาคม 2547

นายสมภาพ มองเคน นิสิตคณะวิทยาศาสตร์

วันที 19-28 พฤษภาคม 2547

นายรวิสุต จรรยพงษ์	นิสิตคณะวิทยาศาสตร์
นายพงษ์ธร โชติเกษมศรี	นิสิตคณะวิทยาศาสตร์
นายชญานิน โชคดีมีบุญ	นิสิตคณะวิทยาศาสตร์

รายชื่อนิสิตแพทย์ฝึกงานจากต่างประเทศ

1. ตุลาคม – พฤศจิกายน 2544

   Dr. Vu Thuy Yen

   แพทย์เวียตนามมาฝึกอบรม

2. มกราคม – กุมภาพันธ์ 2545

   Jost Labout : Erasmus University Rotterdam, The Netherlands ศึกษา

   polymorphism ของ HCV และ genotypes

3. มีนาคม – มิถุนายน 2545

   Ms. CH Geurts Van Kessel :

   Erasmus University Rotterdam, The Netherlands ศึกษา respiratory virus

4. ตุลาคม – พฤศจิกายน 2545

   Ms. CMM Suripatty นักศึกษาแพทย์จาก Erasumus University Rotterdam, The

   Netherlands ศึกษา respiratory virus

5. พฤษภาคม-มิถุนายน 2545

   MS. Sandy Chira : Arcadia USA

   ศึกษา Molceular Biology technique

6. Ms. Leontien Vermeulen

   Ms. Florine van der Vegt

   นักศึกษาแพทย์จาก Erasumus University Rotterdam,

   ศึกษาเรื่อง Viral hepatitis and molecular biology techniques

7. Miss Porn Triratvorakul

   นักศึกษาแพทย์จาก University of Melbourn ในหลักสูตร Advance Medicsl Science

   ทำการศึกษาวิจัยเรื่อง Nitric Oxide กับโรคไข้เลือดออก

เมธีวิจัยที่ทำการศึกษาวิจัยกลุ่ม

ผศ.นพ.พิสิฐ ตั้งกิจวานิช

ภาควิชาชีวเคมี คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ผศ.พญ.ดร. ณัฏฐิยา หิรัญกาญจน์

ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

อ.สพญ.ดร สันนิภา สุรทัตต์

ภาควิชาจุลชีววิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ที่ปรึกษาทุนหลังปริญญาเอก และอาจารย์ใหม่

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นักวิทยาศาสตร์ ที่ปฏิบัติงานของศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ

1. นางสาวอภิรดี เทียมบุญเลิศ หัวหน้าห้องปฏิบัติการ ศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ
2. นางสาวกวีตา เบดี นักวิทยาศาสตร์ ศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ
3. นางสาวจิตติมา ทองมี นักวิทยาศาสตร์ ศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ
4. นางสาวอรอุมา แย้มบางยาง นักวิทยาศาสตร์ ศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ
5. นางสาวนุชนาฏ ถาวรสุข นักวิทยาศาสตร์ ศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ
6. นางสาวปิติรัตน์ บุญสุข นักวิทยาศาสตร์ ศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ
7. นางสาววิลาวัลย์ วิวัฒนทีปะ นักวิทยาศาสตร์ ศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ

ความเห็นของผู้วิจัย

โครงการวิจัยได้ดำเนินไปด้วยดีและทางกลุ่มได้เติบโตขยายใหญ่ขึ้น ทางหน่วยปฏิบัติการวิจัยไวรัสตับอักเสบได้รับการสนับสนุนจากจุฬาลงกรณ์มหาวิทยาลัย ให้เป็นศูนย์เชี่ยวชาญเฉพาะทางด้านไวรัสตับอักเสบ ทางศูนย์มีความสามารถเป็นที่ยอมรับในวงวิชาการและการบริการสังคมทั่วไป เช่นการตรวจไวรัสตับอักเสบให้ศูนย์บริการโลหิตแห่งชาติและภาคเอกชน อย่างไรก็ตามการสร้างนักวิจัยและจรรยาบรรณของนักวิจัยรุ่นใหม่ยังมีความจำเป็นเพื่อสร้างกลุ่มวิจัยและประชาคมวิจัยมีความเข้มแข็ง การได้รับการสนับสนุนจาก สกว. ทำให้ศูนย์มีศักยภาพเพิ่มขึ้นมาโดยตลอด

เอกสารแนบ (ถ้ามี)

ข้อมูลและประวัติย่อของผู้ได้รับทุนวิจัยระดับปริญญาโท/เอก จากโครงการ

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บทคัดย่อหรือบทสรุปของบทความวิชาการหรือเอกสารในลักษณะอื่นๆ ตลอดจนสื่อประเภทต่าง ๆ ที่โครงการได้จัดทำขึ้น

ผลงานอื่น ๆ ที่เกี่ยวข้อง

ในการเผยแพร่ผลงานวิจัยของกลุ่ม ในกลุ่มได้มีนโยบายให้อาจารย์รุ่นใหม่ นิสิตดุษฎีบัณฑิต มหาบัณฑิต ไปเผยแพร่ผลงานวิจัยในกลุ่มวิชาชีพ สมาคมที่เกี่ยวข้องอันจะเป็นประโยชน์อย่างมากต่องานวิจัยประเทศไทย และเป็นการเผยแพร่ชื่อเสียงให้กับคณะกลุ่มวิจัย และ สกว. แทนการจัดประชุมประจำปี ซึ่งจะใช้ค่าใช้จ่ายจำนวนมาก และผู้ร่วมฟังอภิปรายส่วนมากจะเป็นผู้ใกล้ชิด การจัดประชุมของกลุ่มวิจัยได้มีการจัดประจำเดือน มีการแลกเปลี่ยนความคิดเห็นในงานวิจัยอยู่เป็นเนืองนิตย์แล้ว

ผลงานของกลุ่มในการได้รับทุนเมธีวิจัยครั้งที่ 2 นี้ มีผลงานที่เป็นรูปธรรม ในวารสารนานาชาติ ที่ดูภาพรวมแล้วมีทั้งจำนวนมาก และวารสารที่ได้เผยแพร่เป็น วารสารที่มี high impact factor กว่าการได้รับทุนครั้งที่ 1 จะเห็นว่าวารสารที่ลงมี impact factor มากกว่า 3 เช่น J Gein Virol 2 เรื่อง วารสาร Vaccine วารสาร Am J Gastroenterol และวารสารจำนวนมากที่ impactfactor อยู่ 1-3 ที่ได้ลงแล้วหรือตอบรับแล้ว เช่น J Virol Methods, J Gastroenterol, Scan J Infect, Infection, Ann Trop Med Parasitol J Gastroenterol Hepatol, J Med J Gastroenterol Hepatol, J Med primate นับว่าเป็นเชิงคุณภาพประสบความสำเร็จ คุ้มกับทุนที่ได้ลงไป

การศึกษาวิจัยร่วมกันเป็นกลุ่มแสดงให้เห็นถึงศักยภาพของนักวิทยาศาสตร์ นักวิจัยไทยในการศึกษาวิจัยไข้หวัดนกในประเทศไทย โดยทีมงานผู้วิจัยสามารถรวบรวมทีมนักวิจัย นักวิทยาศาสตร์ นิสิตปริญญาโท ปริญญาเอก เข้าร่วมการวิจัยอย่างเร่งด่วนกว่า 20 คน ในการพัฒนาการตรวจวินิจฉัยและถอดรหัสพันธุกรรมของไข้หวัดนกให้มีผลงานเป็นที่ประจักษ์ในสายตาชาวโลก ขณะนี้งานต่าง ๆ ได้ดำเนินไปด้วยดี โดยได้รับการสนับสนุนอย่างดียิ่งขึ้นจากทุนเร่งด่วนจากจุฬาลงกรณ์มหาวิทยาลัย และกำลังดำเนินการขอทุนเพิ่มเติมจากศูนย์พันธุวิศวกรรมแห่งชาติ การสร้างนักวิจัยรุ่นใหม่ ขณะนี้นักวิจัยที่สามารถยืนได้เอง และได้ออกจากกลุ่มแล้ว เช่น รองศาสตราจารย์นายแพทย์วรศักดิ์ โชติเลอศักดิ์ ผู้ช่วยศาสตราจารย์นายแพทย์อิศรางค์ นุชประยูร ผู้ช่วยศาสตราจารย์แพทย์หญิงสุรางค์ นุชประยูร อาจารย์ดังกล่าวสามารถหาทุนและทำวิจัยได้เอง มีประสิทธิภาพและอาจารย์ดร.สันนิภา สุรทัตต์ จะเป็นอีกท่านหนึ่งที่จะมีความก้าวหน้าทางการวิจัยอย่างมากในอนาคต

การสร้างนิสิตปริญญาโท เอก ก็นับว่าประสบความสำเร็จระดับหนึ่งจากการที่แต่เดิมคณะแพทยศาสตร์ จะเน้นเรื่องของสายอาชีพ training มากกว่า ขณะนี้นิสิตปริญญาเอก จบการศึกษาไปแล้ว 2 คน และอีก 1 คน ถึงแม้ว่าจะต้องออกจากการศึกษาผลงานก็สามารถลงพิมพ์ในวารสารเผยแพร่ได้ดี ขณะนี้มีนิสิตปริญญาดุษฎีบัณฑิตเหลืออยู่ 3 คน และวางแผนจะรับเพิ่มเติมอีก สำหรับปริญญามหาบัณฑิตเหลืออยู่มากกว่า 7 คน และวางแผนจะรับเพิ่มเติมอีก สำหรับปริญญามหาบัณฑิตมีหลายคนที่อยู่ระหว่างการศึกษา

การสร้างเครือข่ายกับต่างประเทศประสบความสำเร็จ จะเห็นว่าความมีชื่อเสียงของศูนย์ฯ ทำให้เป็นที่ยอมรับของสถาบันต่างชาติ ส่วนนักศึกษาที่เข้ามา train ในศูนย์ฯมีจำนวนเพิ่มขึ้นตลอด เช่น จากเนเธอร์แลนด์ ออสเตรเลีย

รวมบทคัดย่อ
ผลงานเผยแพร่ระดับนานาชาติ

CLINICAL AND MOLECULAR CHARACTERISTICS OF THAI PATIENTS WITH ACHONDROPLASIA

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Abstract. Achondroplasia is an autosomal dominant disorder characterized by disproportionately short stature, frontal bossing, rhizomelia, and trident hands. Most patients appear sporadically resulting from a *de novo* mutation associated with advanced paternal age. A glycine to arginine mutation at codon 380 (G380R) of the fibroblast growth factor receptor 3 gene (*FGFR3*) was found to be the most common cause of achondroplasia in various populations. We identified and clinically characterized 3 Thai patients with achondroplasia. In all of them, we also successfully identified the G380R mutation supporting the observation that this is the most common mutation in achondroplasia across different ethnic groups including Thai.

INTRODUCTION

Patients with short stature display an extremely long list of differential diagnoses. Achondroplasia is one of them. Clinical manifestations and molecular defects of patients with achondroplasia have been described in various ethnic groups. Here we report three Thai patients with achondroplasia whose molecular abnormalities were successfully identified, providing a specific method for molecular diagnosis of patients and for prenatal diagnosis in families at risk.

MATERIALS AND METHODS

Case reports: Three patients coming to the Genetics Clinic at King Chulalongkorn Memorial Hospital were diagnosed with achondroplasia. Patient 1 was born at term to a 37 year-old G3P2 Thai mother and a 43 year-old unrelated Thai father. Neither the parents nor the two elder sisters of patient 1 were affected. Pregnancy and delivery were uncomplicated. His birth weight was 3,590 g (+1 SD), length 47 cm (-2 SD), and head circumference 38.5 cm (+3 SD). In addition to short stature, physical examination revealed increased upper to lower trunk ratio (2.2:1) (normal 1.7:1), frontal bossing, rhizomelia, trident hands, left hydrocele, and lordosis (Fig 1A). Achondroplasia was diagnosed soon after birth. At 8 months of age, his head circumference was 49 cm (+4 SD). Due to the rapid increase of his head size, a CT scan of the brain was performed revealing hydrocephalus. A ventriculoperitoneal shunt was placed. Developmental assessment by the Gesell Developmental schedule showed a developmental quotient of 73 at the chronological age of 1 year and 8 months. The left hydrocele was surgically repaired at 1 year and 9 months. Polysomnography performed at 2 years and 6 months was normal. At 4 years and 6 months, growth hormone provocative tests by insulin and clonidine showed maximum growth hormone levels of 1.9 and 6.4 ng/ml, respectively, indicating growth hormone deficiency. The IQ test by WISC III revealed verbal IQ, performance IQ and full IQ of 84, 103, 93 respectively at 8 years of age. Radiography of the lumbar spine showed caudal narrowing

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ADULT IMMUNIZATION – A NEGLECTED ISSUE IN SOUTHEAST ASIA

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Abstract. Adult immunization is a neglected and underpublicised issue in Southeast Asia. Vaccine-preventable diseases cause unnecessary morbidity and mortality among adults in the region, while inadequate immunization results in unnecessary costs, including those associated with hospitalization, treatment, and loss of income. Childhood vaccination coverage is high for the EPI diseases of diphtheria, tetanus and pertussis; however, unvaccinated, undervaccinated, and aging adults with waning immunity remain at risk from infection and may benefit from vaccination. Catch-up immunization is advisable for adults seronegative for hepatitis B virus, while immunization against the hepatitis A and varicella viruses may benefit those who remain susceptible. Among older adults, immunization against influenza and pneumococcal infections is likely to be beneficial in reducing morbidity and mortality. Certain vaccinations are also recommended for specific groups, such as rubella for women of child-bearing age, typhoid for those travelling to high-endemicity areas, and several vaccines for high-risk occupational groups such as health care workers. This paper presents an overview of a number of vaccine-preventable diseases which occur in adults, and highlights the importance of immunization to protect those at risk of infection.

INTRODUCTION

Immunization policies in Southeast Asia have been primarily directed towards vaccinating infants and children. With the focus on childhood immunization, the importance of adult immunization has been overlooked, making it a neglected and under-publicised issue in this region. A substantial proportion of vaccine-preventable morbidity and mortality occurs among adults worldwide (Fedson, 1994; Marth, 1997; Bouvet and Micoud, 1997). While many infections predominantly affect younger adults, others occur more commonly among middle-aged and older adults. Inadequate immunization against these infections results in substantial and unnecessary costs, both in terms of hospitalization and treatment, and in lost income (Fedson, 1994).

A range of factors contribute to low immunization levels among adults. (Fedson, 1994; Marth, 1997; Zimmerman and Clover, 1995; Gardner *et al*, 1996). Many adults are not aware of the need for immunization, others are concerned about potential side-effects, while the cost of vaccination and access to vaccines and vaccine delivery services may be a barrier for some. Health care providers may miss opportunities to offer vaccination, and overlook indications based on lifestyle

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LONG-TERM EFFECT OF INTERFERON THERAPY ON INCIDENCE OF CIRRHOSIS AND HEPATOCELLULAR CARCINOMA IN THAI PATIENTS WITH CHRONIC HEPATITIS B

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Abstract. The aim of this study was to assess the long-term effects of interferon (IFN) therapy on the incidence of disease progression to cirrhosis and hepatocellular carcinoma (HCC) in Thai patients with chronic hepatitis B. Sixty-seven patients with hepatitis B e antigen (HBeAg)-positive chronic hepatitis B who received IFN therapy were retrospectively analyzed. The average duration of follow-up was 59.4±30.9 months (ranging from 20 to 119 months). Seventy-two untreated patients with comparable clinical data and mean duration of follow-up served as a control group. During follow-up, 24 (35.8%) treated and 7 (9.7%) untreated patients had a sustained loss of HBeAg. However, none of the treated patients or controls became negative for hepatitis B s antigen (HBsAg). Among treated patients, the response was independent of type and dose of IFN, as well as the presence of steroid priming. In addition, 1 of 24 (4.2%) sustained responders and 6 of 43 (14%) non-responders progressed to cirrhosis whereas 16 of 72 (22.2%) in the control group progressed to such sequelae. The overall incidence of new cirrhosis in sustained responders was significantly lower than in the control group (p=0.04). HCC appeared in 11 cirrhotic patients: 9 (12.5%) in the control group and 2 (4.7%) of the non-responders, whereas none of the sustained responders developed HCC. The average period to detection of HCC was 70.5±12.4 months for non-responders and 65.3±27.6 months for the control group, with no significant differences between these groups. In conclusion, our data suggest that IFN therapy might prevent the progression of cirrhosis and the development of HCC in patients with chronic hepatitis B. These beneficial effects were particularly observed in those who achieved a sustained virological response to treatment.

INTRODUCTION

Hepatitis B virus (HBV) infection is considered a leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) in Southeast Asia, China and sub-Saharan Africa (Lee, 1997). Currently, interferon (IFN) is widely used for treatment of chronic HBV infection in most countries. The aims of IFN therapy are to suppress or eradicate HBV, to diminish necroinflammation and to reduce the risk of cirrhosis and HCC (Perrillo, 1993). Among patients treated for 3-6 months, its therapeutic efficacy rate is approximately 30-40% in terms of virological and histological remission (Wong *et al*, 1993; Hoofnagle and DiBisceglie, 1997). Furthermore, previous studies have suggested that loss of HBeAg in chronic HBV infection, whether due to IFN therapy or occurring spontaneously, is associated with improved clinical outcome (Niederau *et al*, 1996; Lau *et al*, 1997; Fattovich *et al*, 1997). However, it is still unclear whether IFN therapy could prevent the development of cirrhosis and HCC in patients with chronic hepatitis B. Thus, the main aim of the current study was to evaluate retrospectively the long-term beneficial effects of IFN therapy on the incidence of cirrhosis and HCC among Thai patients with chronic hepatitis B.

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Hepatitis D Virus Infection in Thailand: HDV Genotyping by RT-PCR, RFLP and Direct Sequencing

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Abstract

Background: Hepatitis D virus (HDV) is a degenerate RNA virus or virusoid that requires the surface coat of hepatitis B virus (HBV), i.e. hepatitis B surface antigen (HBsAg), in order to become infectious. Three distinct genotypes of the virus have been classified. In this study, HDV genotypes were determined by restriction fragment length polymorphism (RFLP) and direct sequencing. In Thailand, simultaneous HDV/HBV infections are particularly prevalent among intravenous drug users (IVDU).

Patients and Methods: A total of 743 IVDU sera were screened for HBV infection. HBsAg-positive samples were subjected to serological analysis for anti-HDV. RFLP analysis using the endonucleases *Xho* I and *Sma* I was performed on the PCR amplified HDV genome to establish the prevailing HDV genotypes.

Results: 55 sera (7%) had detectable HBsAg; all 55 were subsequently subjected to serological analysis for anti-HDV, 12 (21.8%) of which were positive. Eight (66%) specimens had detectable HDV-RNA by RT-PCR. All polymorphisms were shown to be genotype I, a finding confirmed by direct sequencing. 36 HBsAg-positive sera obtained from the blood bank to serve as controls were negative for anti-HDV.

Conclusion: Our data show that HDV infection is still limited among IVDU and that the pattern of polymorphism closely resembles that of the western HDV genotype I.

Key Words

Hepatitis D virus · Genotype · RFLP · Thailand · Sequencing

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Introduction

Hepatitis D virus (HDV) was first discovered by *Rizzetto* et al. [1] in a patient with chronic hepatitis B virus (HBV) infection. HDV is a single-stranded RNA virus requiring HBV as a helper virus to provide the coat protein. Hence, HDV/HBV coinfection may be associated with a higher rate of fulminant hepatitis as a consequence of acute infection. The superinfection of individuals suffering from chronic HBV infection with HDV can lead to both acute and more progressive chronic liver disease [2]. HDV represents a unique agent in animal virology; it is a very small RNA virus (1.7 kb) with virions of approximately 36 nm, composed of a rod-shaped RNA genome and two related structural nuclear phosphoproteins (HDAg) within a protein envelope consisting of hepatitis B surface antigen (HBsAg) [3]. HDV genotyping has been performed by various methods such as reverse transcription (RT)-PCR and restriction fragment length polymorphism (RFLP), hybridization and direct sequencing [4–6]. There are three major groups: genotype I, genotype II and genotype III. The most common, genotype I, has been isolated from Italy, America, Taiwan, Nauru, France and Lebanon [7–9]. Genotype II has been isolated from Taiwan and Japan [9] and genotype III from Peru and Colombia [10]. There is a divergence of 27–34% in nucleotide sequence and of 30% in amino acid sequence among the different genotypes [11]. This divergence can determine the clinical course of HDV infection and has therefore been the subject of studies on the association between genotypes and clinical outcome of HDV infection [12].

In Thailand, HDV infection has a relatively high prevalence within the IVDU group [13]. The genotype of HDV predominant in Thailand has not yet been reported. In this study we subjected HBsAg-positive sera obtained from IVDU to serological tests for anti-HDV and to RT-PCR for detection of HDV-RNA. Subsequently, the respective genotype was determined by RFLP and confirmed by sequencing.

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STATE-OF-THE-ART

Typhoid fever

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Summary

Typhoid fever, the enteric fever caused by Salmonella typhi, *is a major public health problem. Transmission is by the faecal–oral route, and incidence peaks among school-age children (5–19 years). Since multi-drug resistant* S. typhi *is increasing and typhoid fever causes significant morbidity, immunization may prove relevant for epidemic control. Currently three classes of typhoid vaccine are available: inactivated whole-cell, oral attenuated (Ty21a) and virulence (Vi) polysaccharide vaccines. Because they have fewer side-effects, the latter 2 vaccines are recommended by the World Health Organization. The immune response to immunization comprises secretory intestinal antibodies, circulating antibodies and cell-mediated immunity. Populations at high risk of developing typhoid fever, and hence likely to benefit from vaccination, include military personnel, travellers and school-age children in endemic areas, and clinical microbiology technicians. Several studies on novel genetically engineered oral vaccines are in progress; one dose of these vaccines elicits a satisfactory immune response. The incorporation of typhoid vaccine into a country's immunization programme should be based on local epidemiology, including incidence by age group and in high-risk subpopulations, as well as a cost–benefit analysis of the particular vaccine.*

Introduction

Typhoid fever is an enteric fever caused by *Salmonella typhi*; the less severe syndrome caused by *Salmonella paratyphi* A, B or C is known as paratyphoid fever.

Humans are the only reservoir for *S. typhi*. The world-wide incidence of typhoid fever is estimated at approximately 16 million cases per year, of which 7 million occur in South East Asia; the world-wide death rate exceeds 600 000 per year.[1,2] Although the incidence is low in young infants, it peaks among children aged 5–19 years.[2]

The infection is transmitted by the faecal–oral route via both contaminated food and water; the highest incidence occurs where contaminated water supplies serve a large population.[3,4] In developed countries, improvements in sanitation, water supply and food hygiene have resulted in a declining prevalence, and most recent cases are related to international travel[5] or contact with chronic carriers.[6]

Immunopathogenesis and immunology

The first defence against infection by ingested typhoid bacilli is the acid barrier of the stomach. Most *Salmonella* species have developed an acid tolerance that permits survival at pH4 and above, although they are rapidly killed at pH2.[7] Secreted IgA and intestinal mucus may help prevent the bacilli from reaching the enterocytes lining the intestinal wall.

Typhoid bacilli penetrate the intestinal mucosal epithelium by interacting with the microfold cells (M cells) that cover the ileal Peyer's patches.[8] The organisms are internalized on contact with M cells and may therefore enter the systemic circulation via the submucosal lymphoid tissue. Alternatively, they may induce nonphagocytic cells, including enterocytes, to internalize them and thus allow them easy passage without destroying the cells.[9]

Shortly after invasion of the intestinal mucosa, primary bacteraemia sets in. Hence, the bacilli rapidly reach the reticuloendothelial system, and are engulfed by tissue macrophages in bone marrow, liver, spleen and Peyer's patches. The bacteria induce macrophages and epithelial cells to phagocytose them, as they can survive within these cells but not in polymorphonuclear leukocytes. Polymorphonuclear leukocytes can rapidly kill the bacteria, leaving less than 10% of an initial inoculum,[10] and patients with neutropenia or neutrophil dysfunction are at increased risk of disseminated infection. However, patients with impaired or immature reticuloendothelial function are also at risk.[11] For example, children with sickle-cell anaemia commonly develop bacteraemia and subsequent osteomyelitis following *Salmonella* infection.[12]

Problems and Prevention of Viral Hepatitis in Thailand

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Abstract

To this day, viral hepatitis remains a major public health problem in Thailand. Chronic infection with hepatitis B and C viruses are the leading causes of chronic liver diseases, including cirrhosis and hepatocellular carcinoma (HCC). Outbreaks of hepatitis A virus continue to occur in Thailand, even after several years of consistently declining prevalence rates. Also, the reduction in prevalence of hepatitis D virus infection has been observed among intravenous drug users over the past decade. Hepatitis E virus constitutes a rather unusual cause of sporadic acute hepatitis in Thailand. Highly effective vaccines are currently available for prevention of hepatitis A and B, however, as yet no effective vaccine for hepatitis C is imminent. Following rapid progress in the development of molecular techniques, several new hepatitis viruses have been identified. Among these, Hepatitis G, TT and SEN viruses have recently been described but their significance as to causation of human liver disease has yet to be established. This article reviews the current epidemiology, molecular biology, and strategies aimed at prevention and control of hepatitis virus infection in Thailand emphasizing new developments and recent data obtained from our research studies.

Key word : Hepatitis, Liver Disease, Thailand, Prevention

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Hepatitis B vaccine failure

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Hepatitis B virus (HBV) infection constitutes a major public health problem on a global scale. This is particularly so in Southeast Asia where vertical transmission plays a significant role in the spread of HBV.

Hepatitis B vaccine has proven to be highly effective in preventing the HBV infection. Moreover, this is also the first vaccine shown to be able to effectively prevent the occurrence of cancer, in this context hepatocellular carcinoma.[1] Hence, universal administration of hepatitis B vaccine to neonates constitutes the sole means of efficiently reducing the occurrence of HBV infection and this in turn might eventually lead to the eradication of this disease. However, there have been reports of cases of non-responders as well as vaccine failures upon receipt of the complete course of inoculations.[2,3] We have reviewed the prevalence and pathogenetic mechanisms observed among cases of non-response and vaccine failure.

NON-RESPONDERS

Approximately 5 to 10% of individuals receiving standard hepatitis B vaccination fail to produce protective antibody levels (above 10 mIU/ml).[4,5] This lack of response has been attributed to genetic factors and various defects in the immune reaction. These include: defects in antigen uptake, processing and presentation,[6,6a] T-cell dependent suppression of antibody production, defects of T-cell response as well as T-cell repertoire,[7,7a] and failure of T-cells to assist B-cells in producing anti-HBs.[8,9]

Based on the evidence available, non-responsiveness may be related to genetic factors in that the HLA-linked immune response may also control the response to HBsAg. For example in mice, antibody response has been demonstrated to be major histocompatibility complex (MHC) controlled in that certain strains are non-responders, some show intermediate response, while others demonstrate good response.[10,11,12] The ability to respond is inherited as a dominant and lack of response as a recessive trait.

Research article

Cytochrome P450 2E1 polymorphism and nasopharyngeal carcinoma development in Thailand: a correlative study

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Abstract

Background: Nasopharyngeal carcinoma (NPC) is a rare tumor in most parts of the world but occurs at relatively high frequency among people of Chinese descent. The cytochrome P450 2E1 enzyme (CYP2E1) is responsible for the metabolic activation of nitrosamines, and has been shown to be a susceptibility gene for NPC development in Taiwan [RR = 2.6; 95%CI = 1.2-5.7]. Since there has been only one report of this link, it was decided to investigate the susceptibility of *CYP2E1* to NPC development in other populations. Therefore, the correlation between the *RsaI* polymorphism of this gene and NPC was studied in-patients including Thai and Chinese in Thailand. The present study comprised 217 cases diagnosed with NPC and 297 healthy controls.

Results: Similar to the result found in Taiwanese, a homozygous uncut genotype demonstrated a higher relative risk both when all cases were analyzed [RR = 2.19; 95%CI = 0.62-8.68] or individual racial groups, Thai [RR = 1.51; 95%CI = 0.08-90.06] or Chinese [RR = 1.99; 95%CI = 0.39-10.87]. The ethnicity-adjusted odds ratio is 2.39 with 95%CI, 0.72-7.89.

Conclusions: Though our finding was not statistically significant due to the moderate sample size of the study, similarity to the study in Taiwan with only a slight loss in precision was demonstrated. The higher RR found for the same genotype in distinct populations confirmed that *CYP2E1* is one of several NPC susceptibility genes and that the RsaI minus variant is one mutation that affects phenotype.

Background

Nasopharyngeal carcinoma (NPC) is a rare tumor in most parts of the world, with annual age-standardized incidence rates typically below 1 per 100,000 people/year in both sexes [1]. The tumor occurs most often in Southern Chinese who reside in Guangdong Province, at an incidence rate 30-50 per 100,000 people/year, in contrast with <1 per 100,000 people/year in white Europeans [2,3,4,5]. The disease also occurs at moderate frequencies (3-10 per 100,000 people/year) in several non-

Fibroblast growth factor receptor 3 S249C mutation in virus associated squamous cell carcinomas

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Abstract. An S249C mutation in fibroblast growth factor receptor 3 (*FGFR3*) gene was recently identified in patients with cervical carcinomas (CC). However, its importance in cervical tumorigenesis is still inconclusive. Apart from CC, nasopharyngeal carcinoma (NPC) is the other major virus associated squamous cell carcinoma. We sought to clarify the frequency of the *FGFR3* S249C mutation in 75 primary CC in the Thai population and to determine its prevalence in 69 primary NPC by PCR and restriction enzyme digestion. None of the patients but one NPC showed the enzyme digestion pattern consistent with the mutation. This is the first report demonstrating the role of *FGFR3* in the development of human NPC. This study confirms the low frequency of the *FGFR3* S249C mutation in CC. Nevertheless, the discovery of the mutation, not only in CC as reported by previous studies, but in NPC based on this report, suggests that *FGFR3* may play a significant role in human CC and NPC development.

Introduction

Cervical and nasopharyngeal carcinomas are the two major virus associated squamous cell carcinomas. Cancer of the uterine cervix is one of the most common tumors affecting women worldwide with approximately 470,000 new cases diagnosed annually (1). Virtually all cervical carcinomas examined are positive for human papillomavirus (HPV) (2). Even though HPV is considered an essential cause of cervical cancer, it is certainly insufficient to induce transformation and tumor progression (3). A long latency period between HPV infection and tumor appearance is suggested by the fact that the peak incidence of the disease is observed in females above 40 whereas HPV infection occurs in the 20s. Obviously, one important issue is to identify factors marking the transition of the HPV-containing cells to malignancy.

Recurrent genetic alterations in cervical cancer include losses of heterozygosity of many chromosomal regions, recurrent amplification of a few chromosomal sites, and microsatellite instability (3). Specific genes at these loci, however, still remain to be elucidated. Recently, a mutation, 746C→G (S249C), in a gene encoding fibroblast growth factor receptor 3 (*FGFR3*) was identified in 3 of 12 (25%) cervical carcinomas from the French population (4), making it the most common specific molecular genetic alteration in cervical cancer. However, two more recent articles analyzing a larger number of cervical carcinomas refuted the importance of the *FGFR3* activation in cervical tumorigenesis (5,6).

Nasopharyngeal carcinoma (NPC) is relatively common in South China and Southeast Asia with an incidence of 3 to 10 per 100,000 people/year compared to less than 1 per 100,000 people/year in most parts of the world (7,8). Epstein-Barr virus (EBV) appears to be an important etiological factor for NPC (9,10). Several recurrent genetic alterations in NPC have been identified including losses of heterozygosity of many chromosomal regions, recurrent amplification of a few chromosomal sites, and microsatellite instability (11,12). Because of the similarity between cervical cancer and NPC as to their ubiquity in Thailand, their virus associated tumorigenesis, and their histopathology, we sought to clarify the role of the *FGFR3* S249C mutation, the only mutation identified in the *FGFR3* gene in cervical cancers to date, in a large sample of cervical carcinomas in the Thai population and to determine its role in nasopharyngeal tumorigenesis.

Materials and methods

Having obtained informed consent, slides of paraffin-embedded dissected tissues from 23 cervical carcinoma patients, collected between 1997 and 2000, were washed with xylene solution followed by 100%, 95% and 70%

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Key words: cervical cancer, nasopharyngeal cancer, fibroblast growth factor receptor

A REVIEW OF Hib EPIDEMIOLOGY IN ASIA

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Abstract. Meningitis due to an invasive *Haemophilus influenzae* type b (Hib) infection, has been previously perceived to be relatively uncommon in Asia. However, the incidence of disease and its impact may have been underestimated. In addition to a lack of microbiological facilities in some hospitals, difficulties in culturing the organism and the widespread use of antibiotics may have hidden the true incidence of the disease in some countries. Furthermore, the reported disease burden probably underestimates the incidence of Hib pneumonia.

The epidemiology of invasive Hib disease for various Asian nations is reviewed in this paper. Hospital-based studies show that Hib is a major cause of bacterial meningitis and/or pneumonia in the Philippines, India, Thailand, Malaysia, Indonesia and Vietnam. Singapore and Hong Kong have a low incidence of infection compared with Western and other Asian nations. This low incidence is not due to a higher level of natural protective antibodies, but may be related to an interaction between environmental and genetic factors. Therefore the widespread belief that Hib infection is unimportant in Asia does not refer to Asia as a whole and possibly to Chinese patients only, and failure to recognize this has serious implications.

The inclusion of Hib vaccine in the routine infant immunization schedule in many industrialized nations has significantly reduced the incidence of invasive disease. Recent studies have shown Hib vaccination is also effective in preventing invasive disease in children in developing countries.

While population-based data may be required to confirm the need for public-funded infant Hib immunization in Asia, its introduction in countries with a high incidence of Hib meningitis and/or pneumonia has the potential to significantly improve pediatric health and survival.

INTRODUCTION

Haemophilus influenzae type b (Hib) causes serious bacterial infections in children including meningitis, pneumonia, epiglottitis, septicemia and septic arthritis (Shapiro and Ward, 1991). Before the introduction of Hib vaccines, it was the major cause of childhood bacterial meningitis in industrialized nations.

The epidemiology and the clinical pattern of Hib disease in developing countries differs from that seen in industrialized nations. Developing countries have a higher incidence of the disease, and it occurs in younger children, with most cases occurring before 12 months of age (Funkhouser *et al*, 1991; Bijlmer, 1994). In developing countries, pneumonia is a more common manifestation of infection than meningitis (Greenwood, 1992).

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QUADRENNIAL REVIEW

Epidemiology and prophylaxis of viral hepatitis: A global perspective

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Abstract

Background: Viral hepatitis with various forms of acute and chronic liver disease as potential and ultimately fatal sequelae presents a public health problem worldwide.

Methods: Recent published reports on the global epidemiology and prophylaxis of viral hepatitis were reviewed.

Results: With the advances in novel technologies, eight distinct types of hepatitis virus have been described: Hepatitis A, B, C, D, E, G, TT and SEN viruses. Hepatitis A and E viruses are transmitted by the fecal-oral route and do not induce a chronic carrier state. Due to major changes in epidemiology of hepatitis A virus their significance is more pronounced in areas of intermediate endemicity. Since the available hepatitis A vaccine is rather expensive, cost-benefit studies should be performed with emphasis on the area under consideration or specialized vulnerable groups. Parenterally transmitted hepatitis B and C viruses are major causes of chronic liver disease, including cirrhosis, hepatocellular carcinoma and end-stage liver failure. Hepatitis D virus is unable to replicate on its own, it requires an established hepatitis B virus infection to be able to replicate. Since its introduction, hepatitis B vaccine has been widely used leading to a significant decrease in HBV infection in countries with universal vaccination. Hepatitis G and TT viruses have been characterized within the latter part of the past decade but their significance as to the causation of human liver disease has yet to be elucidated. Likewise, the precise impact of the most recently described SEN virus isolated from patients with post-transfusion hepatitis awaits further studies.

Conclusions: In the course of this review, we present the situation and focus on research activities emphasizing epidemiology and prevention of the various forms of viral hepatitis.

Keywords: epidemiology, prophylaxis, hepatitis virus, prevention, vaccine, global.

INTRODUCTION

Viral hepatitis has remained a major public health problem worldwide. With the advance in technologies, eight distinct types of hepatitis viruses have been described so far: Hepatitis A, B, C, D, E, G, TT and SEN viruses. In this article, we review the present situation on epidemiology and prophylaxis of various forms of viral hepatitis in the global perspective.

HEPATITIS A

Hepatitis A virus (HAV) is a small, nonenveloped RNA virus of the genus Hepatovirus, family picornaviridae, usually transmitted by the fecal-oral route through interpersonal contact. Hepatitis A virus is thermostable and acid resistant. Since the virus lacks a lipid envelope, it is also resistant to bile lysis. This latter capacity allows efficient fecal-oral transmission. The likelihood of showing symptoms related to HAV infection is related to the age of the patient. Most infections in children below the age of 6 years are asymptomatic, whereas

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Infection with hepatitis C virus among intravenous-drug users: prevalence, genotypes and risk-factor-associated behaviour patterns in Thailand

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Hepatitis C virus (HCV) infection, a major problem worldwide, is usually transmitted parenterally or by use of contaminated needles among intravenous-drug users (IVDU). In a cross-sectional study, demographic data were collected and behaviour patterns investigated in interviews with 453, consenting IVDU. Blood samples were collected from each interviewee and checked for anti-HCV antibodies and, in a PCR-based assay, for the RNA of HCV. Almost all (92.5%) of the IVDU investigated were found positive for anti-HCV and/or the viral RNA. Most (73.5%) of those positive for HCV RNA were found to be infected with genotype 3a alone, the rest being infected with 1b (17.9%), 6a (3.5%), 3b (1.4%), 1a (1.0%), or both 3a and 6a (2.1%) or having non-typable infections (0.6%). Curiously, 26.0% of those who appeared seronegative for anti-HCV were positive for HCV RNA. The longer an interviewee had been using intravenous drugs, the more likely he or she was to be infected with HCV. Among the IVDU, the sharing of needles, syringes and/or other drug-related paraphernalia appeared to be the behaviour carrying the highest risk of HCV infection, giving an adjusted odds ratio and (95% confidence interval) of 4.84 (1.88–12.43). Programmes of needle and syringe exchange should probably be implemented among IVDU in Thailand and elsewhere, to slow the transmission of HCV.

Hepatitis C virus (HCV) is a single-stranded RNA virus of the family *Flaviviridae* and closely related to the viruses causing dengue, Japanese B encephalitis and yellow fever. It has been estimated that 170 million people — 3% of the global population — are infected with HCV (WHO, 1997). The results of phylogenetic-tree analysis (Simmonds *et al.*, 1994) indicate that HCV has at least six main genotypes and three of these (genotypes 1b, 3a and 6) are prevalent in Thailand (Theamboonlers *et al.*, 2000). HCV is transmitted parenterally or by direct, percutaneous exposure to infectious materials, such as through blood transfusion from infectious donors (a particular problem in countries where blood donations are not screened for HCV; Chinchai *et al.*, 2001), the sharing of contaminated needles among intravenous-drug abusers (Alter and Moyer, 1998), and needle-stick injuries among healthcare workers (Kiyosawa *et al.*, 1991). Approximately 2.3 million–4.7 million HCV infections may occur each year as the result of the use

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THE PREVALENCE AND GENOTYPES OF HEPATITIS C VIRUS INFECTION AMONG DIFFERENT GROUPS OF DRUG ADDICT AND BLOOD DONORS

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Hepatitis C virus infection, which can cause chronic liver diseases, cirrhosis and hepatocellular carcinoma, is still a major problem worldwide, with approximately 170 million people already infected. In order to understand the epidemiology and route of transmission, we examined the prevalence and genotypes of hepatitis C virus among different groups of drug addicts and blood donors in Thailand. There were intravenous drug users (n=134), methamphetamine users (n=100), inhalation drug users (n = 19) and alcoholics (n=50). The prevalence of hepatitis C in blood donors was determined by screening among new donors (n=66,340) in 2000. One hundred and seventy nine blood donors with positive antiHCV were randomly selected for HCV-RNA and genotyping. HCV-RNA was performed by nested RT-PCR from the 5' noncoding region. The genotypes assay was based upon analysis of amplified sequences from the 5' NCR and RFLP (Davidson et al. 1995 and Mellor et al 1996). The results showed that there was a higher rate of HCV infection in the IVDU group (70%) compared to the other drug addict groups (12-21%) and new blood donors (0.98%).
Based on our data and considering that the virus is blood borne, the risk factor for HCV transmission in the IVDU group is contaminated blood transmitted from person to person by the sharing of needles. HCV genotype 3a, 1b and 6a are the predominated genotypes in Thailand.

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A 7-YEAR LOOK-BACK INVESTIGATION ON THE RISK OF PROVIDER-TO-PATIENT TRANSMISSION OF HEPATITIS C VIRUS

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Context: Currently, it is not known how often the hepatitis C virus (HCV) is transmitted from infected health-care workers to patients during medical care and what is the potential public health impact of these incidents.
Objective: To determine the rate of provider-to-patient transmission of HCV among former patients of an HCV positive gynecologist, who infected one of his patients during a caesarean section.
Design: Retrospective epidemiological study supported by molecular virological analyses.
Setting: A department of gynecology and obstetrics in a German Academic Teaching Hospital.
Patients: A total of 2,907 woman, who had been operated on by the HCV infected gynecologist between July 1993 and March 2000.
Results and conclusions: Of the 2,907 women affected, 78.6% could be screened for markers of HCV infection. Seven of these former patients were found to be HCV infected. Phylogenetic analysis of HCV sequences from the gynecologist and the women did not indicate that the virus strains were linked. Therefore, no further iatrogenic HCV infections caused by the gynecologist could be detected during the look-back investigation. The resulting overall HCV transmission rate was 0.04% (1/2,286). The observed HCV transmission rate was within the range predicted previously by model-based probability calculations. Although further look-backs among former patients of other surgical specialities are needed, our findings support the notion that provider-to-patient transmission of HCV is a relatively rare event and might provide a factual basis for future recommendations on the management of HCV-infected health care workers.

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Management of Chronic Hepatitis B and C Virus Infections

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Abstract. Hepatitis B and C virus (HBV and HCV) infections present an important health problem causing significant morbidity and mortality on a worldwide scale. The younger the subjects infected, the higher the risk predisposing to progression towards chronic infection. Treatment of chronic HBV and HCV infections is aimed at reducing hepatic inflammation and thus improving the symptoms, decreasing the likelihood of long-term sequelae such as hepatocellular carcinoma, and increasing the survival rate. Interferon accelerates the spontaneous course of chronic HBV infection in children with greater disease activity and lower levels of replication. There is limited information on the use of lamivudine and its long-term benefit in children with chronic HBV infection. The response of combination therapy with IFN and ribavirin in children with chronic HCV infection is still under investigation. The long-term clinical and virological effects of various drugs used in chronic HBV and HCV infections on children remain to be evaluated. **[Indian J Pediatr 2002; 69 (2) : 149-154]**

Key word : *Chronic hepatitis; HBV; HCV; Children*

Hepatitis B and C virus infections can cause chronic liver diseases such as chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Those infected with either virus in early childhood are at risk of developing virus related HCC at a younger age than those infected in adulthood.

HEPATITIS B VIRUS

Hepatitis B virus (HBV) is a DNA-containing 42-nm Hepadnavirus. HBV infection presents an important health problem causing significant morbidity and mortality on a worldwide scale. There are at least 350 million carriers of HBV worldwide.[1] Approximately 25-30% of these carriers who have acquired HBV infection either during infancy or early childhood will eventually succumb to one or other of the potentially ensuing fatal sequelae, such as liver failure, chronic hepatitis, cirrhosis, and HCC. At least 1 million chronically HBV infected individuals die each year from those sequelae.[2] The global prevalence of chronic HBV infection varies from high (≥8%) in Africa, Asia, and the Western Pacific to intermediate (2-7%) in Southern and Eastern Europe, and low (<2%) in Western Europe, North America, and Australia. Around 75% of chronic HBV carriers are populations of Asian and the Western Pacific regions.[3]

Modes of HBV transmission include vertical (perinatal) and horizontal ones, such as transmission by multiple use of needles or instruments not properly sterilized, transfusion of unscreened blood, or sexual contact. However the most significant route of transmission in Asian countries is vertically transmission, which in most cases leads to be a chronic carrier stage.

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NATURAL HISTORY OF CHRONIC HBV INFECTION

The course of chronic HBV infection can be divided into three phases based on virus-host interactions resulting in a variety of hepatic lesions. The first phase, the so-called 'immuno-tolerant phase' is characterized by high levels of viral DNA in serum, expression of HBeAg, and no or minimal hepatic inflammation and hence, an asymptomatic course of the disease. This phase may persist for two or three decades.

The second, so-called immune clearance or 'active' phase is characterized by intermittent or continuous hepatitis of varying degrees of severity. Exacerbations are the result of intermittent efforts by the host's cell-mediated immune response to eliminate replicating HBV. Seroconversion to anti-HBe expression may occur during this phase. In Southeast Asia, this second phase is usually observed in individuals between 20-40 years of age. In the course of the first two phases, HBV is actively replicating. The third phase is the 'inactive' phase, during which viral concentrations are low and thus, there is minimal hepatic inflammation. During this phase, although some of the infected hepatocytes have been eliminated, HBV DNA can become integrated into the host genome and hepatocytes containing HBsAg gene continue to express the antigen. The severity of symptoms during this phase depends on the previous phase.

The risk of chronic infection is related to two major factors including the age at which infection occurs and the capacity of the host's immune response to eliminate the virus from the hepatocytes. As a consequence of immune

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SHORT COMMUNICATION

Declining Hepatitis A Seroprevalence Among Medical Students in Bangkok, Thailand, 1981-2001

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SUMMARY The severity of clinical symptoms following hepatitis A virus (HAV) infection is age dependent. Hepatitis A in children is mostly an asymptomatic disease while adolescents and adults usually show symptoms of clinical hepatitis. Improved personal hygiene and environmental sanitation has led to a decline in natural immunity acquired in childhood, creating a population of susceptible adults. In the past decade, the incidence and prevalence of hepatitis A disease in Thailand have decreased significantly. In this study, we used enzyme-linked immunosorbent assay to determine the prevalence of anti-HAV antibodies among medical students at two different time points in 1996 and 2001. We then compared these results with data from previous studies in 1981 and 1992. The seroprevalence was 73.01%, 30.23%, 16.67% and 6.67% in 1981, 1992, 1996 and 2001, respectively. A significant decline has happened over the past two decades ($p < 0.001$). Considering the decreasing immunity to HAV in the younger generations, more cases of symptomatic HAV infection could be anticipated. Further seroprevalence studies in other adolescence groups from different socioeconomic status are needed to elucidate the current situation of HAV infection in the young generation more comprehensively and to develop an appropriate prevention program.

Hepatitis A virus (HAV) is a small, non-enveloped RNA virus of the Hepatovirus genus, in the *Picornaviridae* family. Transmission happens usually via the fecal-oral route through contaminated food and water. Because the virus has no lipid envelope, it resists biliary lysis, therefore, allowing efficient fecal-oral transmission. The likelihood of developing clinical hepatitis after HAV infection depends on the age of the patient. Most infections in children aged under 6 years are asymptomatic, whereas those in older children and adults are usually symptomatic, with jaundice occurring with increasing age.[1,2] However, the hepatitis A virus has just a single serotype and long-lasting immunity develops after infection. Improvement of personal hygiene and environmental sanitation has led to a decline in the number of persons with natural immunity against the virus. This leads to an increasing number of susceptible adults within the population.

In the past decade, the incidence and prevalence of HAV infection in Thailand have significantly decreased. Over the past 25 years, there has been a shift in the epidemiology of hepatitis A in Thailand, from high to moderate endemicity. The prevalence of HAV infection has fallen especially among the younger age groups.[3] This resulted in a growing population of susceptible adolescents and adults, which are the groups more likely to become symptomatic when infected with HAV.

Medical students represent a group within the younger generation. The data on hepatitis A seroprevalence in this group can help reflect the status of HAV infection in adolescents and young adults. In 1982 Viranuvatti *et al.*[4] reported that anti-HAV antibodies were

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Research article

Serological evidence of herpesvirus infection in gibbons

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Abstract

Background: Herpesviruses are not only infectious agents of worldwide distribution in humans, but have also been demonstrated in various non-human primates as well. Seventy-eight gibbons were subjected to serological tests by ELISA for herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), Epstein-Barr virus (EBV) and cytomegalovirus (CMV).

Results: The prevalence of IgG antibodies against HSV-1, HSV-2, EBV and CMV was 28.2%, 28.2%, 14.1% and 17.9%, respectively.

Conclusions: Antigenic cross-reactivity is expected to exist between the human herpesviruses and gibbon herpesviruses. Gibbons have antibodies to human herpesviruses that may reflect zoonotic infection with human herpesviruses or infection with indigenous gibbon herpesviruses. Therefore, it is difficult to draw concrete conclusions from serological studies alone. Identification should be based on further isolation and molecular characterization of viruses from seropositive animals.

Background

Gibbons (*Hylobates spp.*) have become valuable animals for zoological, medical and psychological research. Their small size (the smallest of the anthropoids), ease of handling and maintenance in captivity and their close phylogenetic relationship to humans represent only a few of their desirable characteristics as laboratory animals. Gibbons are found throughout the tropical rainforest of South and Southeast Asia, including Thailand, Malaysia and Indonesia. Illegal pet trade is the main cause of the decreasing gibbon population in Thailand. Since gibbons were categorized as a conserved species in Thailand, hundreds of appropriated and abandoned animals have been handed over to the authorities of the Royal Forest Department (RFD). An infectious disease screening process is necessary to interrupt the spread of diseases, including herpesvirus infection. Little is known regarding natural or experimental herpesvirus infections in this interesting arboreal primate and reports of outbreaks or natural disease are still limited. Hence, screening is required to prevent the spread of infectious diseases, including herpesvirus.

Herpesviruses have been isolated from a wide variety of mammalian and non-mammalian species. The eight human herpesviruses, herpes simplex virus type 1 and 2 (HSV-1 and -2), varicella-zoster virus (VZV), Epstein-Barr

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ORIGINAL ARTICLE

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Plasma endothelin-1 levels in patients with biliary atresia: possible role in development of portal hypertension

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Abstract ***Background***: Biliary atresia (BA) is a severe neonatal liver disease characterized by progressive extrahepatic biliary tract and intrahepatic inflammatory process. Hepatic fibrosis and portal hypertension (PH) still occur despite the disappearance of jaundice following successful hepatic portoenterostomy. Endothelin-1 (ET-1) is a potent vasoconstrictor and has been reported to stimulate hepatic collagen synthesis. The aim of this study was to demonstrate the potential role of ET-1 in the pathogenesis of the progressive inflammation, fibrosis and PH in BA. ***Methods***: Thirty pediatric patients with biliary atresia post-hepatic portoenterostomy and 12 healthy children were examined. The ET-1 level was determined by commercially available enzyme-linked immunosorbent assay kits. ***Results***: Endothelin-1 levels were elevated in the patients compared with those of the controls (5.45 ± 3.34 vs. 2.74 ± 2.17 pg/ml, $P = 0.01$). Moreover, patients with PH also had greater levels of ET-1 than those without PH (6.73 ± 3.27 vs. 3.26 ± 2.2 pg/ml, $P = 0.004$). Patients with abnormal transaminase enzymes had significantly higher ET-1 levels than those with normal enzymes (6.43 ± 3.33 vs. 3.17 ± 2.1 pg/ml, $P = 0.01$). In the jaundice-free group, endothelin-1 levels were elevated in the patients with PH compared with those without PH (5.93 ± 2.15 vs. 2.88 ± 2.1 pg/ml, $P = 0.02$). ***Conclusions***: Our findings showed elevation of plasma ET-1 levels in patients with BA, especially in those with PH. ET-1 levels were also higher in patients with elevated transminase enzymes as well as in the jaundice-free group with PH. ET-1 might play a role in the pathogenesis of the progressive inflammation, fibrosis and PH in BA.

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Keywords Endothelin-1 · Biliary atresia · Portal hypertension

Introduction

Biliary atresia (BA) is a severe neonatal liver disease resulting from a sclerosing cholangiopathy of unknown etiology. Hepatic fibrosis and portal hypertension (PH) still occur despite the disappearance of jaundice following successful hepatic portoenterostomy. The mechanisms responsible for increased collagen production and hepatic fibrosis in BA are still obscure. Ramm and colleagues demonstrated that hepatic stellate cells (HSCs) played roles in the production of type I collagen leading to hepatic fibrosis in patients with BA [1]. They also showed that the hyperplastic bile duct epithelial cells, HSCs, and hepatocytes were the source of the profibrogenic cytokine, transforming growth factor- β_1 (TGF- β_1). However, the mechanisms involved in HSCs activation and bile duct proliferation are still elusive. The increased portal pressure results from an increase in vascular resistance and an elevated portal inflow. The elevated portal pressure leads to the formation of portosystemic collaterals and esophageal varices in an effort to decompress the portal venous system.

The endothelium can initiate vasoconstriction through the release of diffusible vasoconstrictor substances. The stimuli for endothelial-dependent contractions include hypoxia, several neurohumoral mediators (ADP, serotonin and acetylcholine) and physical stimuli (pressure and stretch) [2, 3, 4, 5]. Endothelial-derived contracting factors such as endothelin (ET) are powerful vasoconstrictor substances released from the endothelium and can affect both vascular smooth muscle cell tone and structure [6, 7, 8]. Endothelins consist of a family of contractile peptides (ET-1, ET-2 and ET-3) made up of 21 amino acids [9]. ET-2 and ET-3 differ from ET-1 at the two and six amino acid residue positions, respectively. Endothelins are produced by

RESEARCH NOTE

PREVALENCE AND GENOTYPES OF HEPATITIS C VIRUS INFECTION AMONG DRUG ADDICTS AND BLOOD DONORS IN THAILAND

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Abstract. Hepatitis C virus (HCV) is an infectious agent that has the potential to cause chronic liver disease, cirrhosis and hepatocellular carcinoma. We determined the prevalence and genotypes of HCV infection among groups of drug addicts: intravenous drug users (n = 134), methamphetamine users (n = 100), inhaled-drugs users (n = 19) and alcoholics (n = 50); a group of blood donors acted as a control. The control group consisted of 179 randomly-selected anti-HCV positive samples : these were subjected to HCV RNA screening and genotyping. The anti-HCV test was performed by ELISA; HCV RNA screening was by nested RT-PCR that employed primers from the 5′ noncoding region. The genotype assay was based upon analysis of the 5′ NCR amplified sequences and RFLP. Hepatitis C virus was highly prevalent among all groups of drug addicts (12-70%). In 2000, among the new blood donors (n = 66,340) at the National Blood Center, Thai Red Cross, anti-HCV prevalence amounted to 0.98%. The HCV genotype distribution showed that the most prevalent genotype was 3a, followed by 1b and 6a. Our data demonstrated the very high prevalence of HCV infection in IVDUs, a finding that is consistent with the blood-borne nature of the virus. In order to curb HCV infection, a determined effort to educate both the general population and high-risk groups is required; such a program of education would address both general and particular methods of transmission, especially the use of non-sterile needles etc.

Hepatitis C virus (HCV) infection, which can cause chronic liver diseases, cirrhosis, and hepatocellular carcinoma, is a major problem worldwide: approximately 170 million people are already infected and 3-4 million cases of new infection are expected every year (World Health Organization, 1999). HCV is transmitted parenterally or by direct percutaneous exposure to infectious materials, such as contaminated blood products. This is especially true in countries where donated blood is not screened for HCV and the sharing of contaminated needles by intravenous drug users (IVDVs) is common. In order to understand the epidemiology and route of transmission of HCV, we studied the prevalence and genotypes of hepatitis C virus among groups of drug addicts and blood donors in Thailand. HCV can be classified by phylogenetic tree analysis as at least 6 genotypes, of which 1b, 3a and 6 are prevalent in Thailand (Simmonds *et al*, 1993; Mellor *et al*, 1995).

The study protocol was approved by the Ethics Committee of the Ministry of Public

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Reactogenicity and immunogenicity of reduced antigen content diphtheria–tetanus–acellular pertussis vaccine (dTpa) administered as a booster to 4–6 year-old children primed with four doses of whole-cell pertussis vaccine

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Abstract

A trial to compare the reactogenicity and immunogenicity of a reduced antigen content diphtheria–tetanus–acellular pertussis (dTpa) vaccine with diphtheria–tetanus–whole-cell pertussis (DTPw) vaccine was conducted in Thailand. Three hundred and thirty children aged 4–6 years, primed with four doses of DTPw, received a single injection of either dTpa or DTPw. There was a significantly lower incidence of local and general reactions following dTpa than DTPw ($P < 0.001$). One month after vaccination, 99.4 and 100% of all subjects had protective anti-diphtheria and -tetanus titers, respectively. The vaccine response rate to pertussis antigens was similar in both groups, with 96.9% versus 92.5% for anti-pertussis toxin (PT), 96.9% versus 97.5% for anti-filamentous hemagglutinin (FHA) and 95.1% versus 90.8% for anti-pertactin (PRN) in the dTpa and DTPw groups, respectively. For anti-BPT, the vaccine response in the dTpa group was 29.6% versus 94.4% for DTPw. In conclusion, the dTpa vaccine was as immunogenic and significantly better tolerated than DTPw. The new dTpa vaccine could improve coverage for routine booster vaccination in children and provide a good replacement for DTP vaccines at 4–6 years of age.

Keywords: Immunogenicity; Reactogenicity; Booster; DTPa

1. Introduction

The World Health Organization (WHO) has implemented childhood immunization programs against diphtheria, tetanus and pertussis world-wide since 1974 [1]. However, concerns have been raised about the frequency of local and systemic reactions, as well as the potential relationship between the whole-cell pertussis vaccine and encephalopathy in countries such as Japan, Germany, Italy, Sweden and UK [2–9]. This has led to the development of acellular pertussis vaccines that have been demonstrated to be less reactogenic than whole-cell vaccines [10–12]. Rare and serious adverse events associated with pertussis vaccination are also less frequent after administration of diphtheria–tetanus–acellular pertussis (DTPa). Furthermore, a number of DTPa vaccines have shown to be highly efficacious against the disease [13–15]. Presently, acellular pertussis vaccines are licensed for infant immunization in many countries such as the United States, most European countries, Australia, Latin America and the Asia–Pacific region [16–18].

DTP vaccines display a somewhat increasing reactogenicity with subsequent doses and age [13,19–21]. Therefore, a vaccine with reduced antigen content (dTpa) has been investigated to enable booster vaccination against diphtheria, tetanus and pertussis in one combination for children older than 10 years, adolescents and adults rather than using dT boosters alone. The purpose of this study was to compare the reactogenicity and immunogenicity of reduced antigen content vaccine (dTpa) with a dose of

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Prevalence of canine coronavirus and parvovirus infections in dogs with gastroenteritis in Thailand

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ABSTRACT: Canine coronavirus (CCV) and canine parvovirus type 2 (CPV-2) are the causative agents of gastroenteritis in dogs. Seventy fecal samples from dogs with signs of gastroenteritis (vomiting and diarrhea), twenty-five fecal samples from healthy dogs and one CPV-2 vaccine strain were amplified by semi-nested polymerase chain reaction (PCR) and semi-nested reverse transcriptase polymerase chain reaction (RT-PCR), aimed at specifically studying the gene encoding the most abundant capsid protein VP2 of CPV-2 and spike protein of CCV. The specificity of the CCV RT-PCR product was evaluated by sequencing. Positive specimens comprised 44 samples (62.8%) and 9 samples (12.8%) for CPV-2 and CCV, respectively. In nine CCV positive samples, seven displayed co-infection between CCV and CPV-2. Our CCV sequence (AF482001) showed a 94.9% nucleotide identity to CCV reported in GenBank accession number D13096. High prevalence of CCV and CPV-2 infections was found in 1–2 month- and 3–6 month-old dogs, respectively. Molecular biology of these viruses is important primarily for epidemic control and preventative measures.

Keywords: canine coronavirus; canine parvovirus type 2; gastroenteritis; PCR

Canine viral enteritis should be suspected in dogs with an acute onset of vomiting and diarrhea, especially in puppies and where several animals are affected simultaneously. To date, four viruses have been identified as the essential causes of severe enteritis in dogs: Canine Parvovirus, Canine Coronavirus, Canine Rotavirus and Canine Distemper Virus (Pollock and Carmichael, 1983). Electron microscopic (EM) examination of fecal suspensions or isolation in tissue cultures are the most commonly used techniques for diagnosis of the infection in dogs. Recently, polymerase chain reaction (PCR) has increasingly been employed for detection of pathogens, especially when present at very low titers. The PCR is characterized by high sensitivity, specificity, and rapidity, thus becoming widely used for detecting various microorganisms.

Initially, the only prophylactic intervention available against canine parvovirus type 2 (CPV 2) comprised inactivated or live attenuated feline panleukopenia virus vaccines which proved largely ineffective. At a later, vaccines derived from live attenuated CPV-2 become available. Vaccination with an inactivated canine coronavirus (CCV) vaccine can significantly reduce not only viral replication, but the occurrence of clinical disease following a virulent CCV infection (Fulker *et al.*, 1995). In Thailand, the data available on CCV and CPV-2 infection are limited. The objective of the present study is to evaluate the prevalence of CCV and CPV-2 infections in gastroenteritic dogs by using semi-nested RT-PCR and semi-nested PCR to detect CCV RNA and CPV-2 DNA, respectively, in fecal specimens derived from gastroenteritic dogs and healthy dogs.

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Plasma Endothelin-1 in Infants and Young Children with Acute Bronchiolitis and Viral Pneumonia

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SUMMARY Respiratory syncytial virus (RSV) infections that occur during the first three years of life have been demonstrated to be associated with the development of childhood asthma. The mechanism of virus-triggered airway inflammation is not fully understood. Endothelin-1 is a potent bronchoconstrictor involved in many diseases including respiratory tract infections. Infants and young children diagnosed with either viral pneumonia or acute bronchiolitis, their age ranging between 2 months and 3 years, were recruited into this study. Nasopharyngeal aspirates were taken for detection of respiratory virus by antigen immunofluorescence stain, RT-PCR analysis and viral culture. Plasma endothelin-1 (ET-1) was measured by using a commercially available enzyme-linked immunosorbent assay (ELISA). Ten of the nineteen infants and children (52%) were positive for RSV infection, one co-infected with Influenza A. Nine infants (90%) were positive for RSV subtype A. There was only one infant with subtype B. One of the RSV negative individuals was positive for Influenza A. In addition, we recruited 10 patients without chronic underlying or respiratory tract illness as controls. ET-1 levels were significantly increased in RSV infection compared to the controls (3.6 ± 1.2 and 1.2 ± 1 pg/ml, respectively ($p < 0.05$). In conclusion, infants and young children who are infected with RSV have an increase in circulating plasma endothelin-1. This in turn may contribute to the subsequent development of childhood asthma.

Acute bronchiolitis and viral pneumonia consume a substantial amount of resources of primary healthcare systems throughout the world.[1-3] Respiratory syncytial virus (RSV), a major pathogen within the paramyxovirus family, causes severe lung disease in young children as well as immunocompromised individuals. It is the most frequent cause of acute bronchiolitis and pneumonia in infants and young children requiring hospitalization.[4-5]

Studies done during the past few decades have expanded our knowledge extensively regarding the specific mechanisms involved in the pathogenesis of RSV bronchiolitis and subsequent chronic obstructive airway disease. It is known that RSV bronchiolitis and subsequent development of asthma may be triggered by Th2-type cytokines.[6] The airway's epithelial cells are the primary target cells for RSV infection. A growing body of evidence suggests that the epithelium is not only a physical barrier, but also has the potential to synthesize a variety of cytokines, e.g. interleukin-8 (IL-8), granulocyte macrophage-colony stimulating factor (GM-CSF) and transforming growth factor (TGF). During acute RSV infection the immune response may induce long-lasting detrimental effects, thereby contributing to post bronchiolitis wheezing.[7-9]

Endothelin-1 (ET-1) is another important cytokine with a major impact on asthmatic patients. It is an endothelial regulatory peptide present in pulmonary tissue where it exerts several biological effects both on bronchial and vascular smooth

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Oral Medicine

Hepatitis C virus infection in Thai patients with oral lichen planus

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OBJECTIVE: Many studies focusing on the association between hepatitis C virus (HCV) infection and oral lichen planus (OLP) have been conducted. Diversities of geographical locations could be a major factor influencing the prevalence of HCV. This study was aimed to define whether there was a relationship between the OLP and HCV infection in Thailand.

MATERIALS AND METHODS: Serum samples of 60 patients (with OLP) and 60 controls (without OLP), whose age and gender were matched, were respectively screened for anti-HCV by ELISA (third generation), and reverse transcription polymerase chain reaction (RT-PCR) for HCV-RNA.

RESULTS: We found five patients (8.33%) with OLP infected with HCV: three patients were positive for both anti-HCV and HCV-RNA; one patient was only positive for anti- HCV; and one patient was only positive for HCV-RNA; whereas all the controls were negative for both anti-HCV and HCV-RNA ($P = 0.029$). Three of five cases of OLP with HCV infection had histories of blood transfusions over 10 years ago.

CONCLUSION: The present study reports a small, but statistically significant high prevalence of HCV infection among patients with OLP, although the underlying mechanism still remains unknown.

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Keywords: hepatitis C virus; oral lichen planus; anti-HCV; HCV-RNA

Introduction

Lichen planus (LP) is relatively a common, chronic mucocutaneous disease of unknown etiology (Regezi and Sciubba, 1993). During the last two decades, several reports proposed a possible association between LP and chronic liver disease (CLD) (Ayala *et al*, 1986; Cottoni *et al*, 1988; Gruppo Italiano Studi Epidemiologici in Dermatologia (GISED), 1990; Rebora, Robert and Rongioletti, 1992), especially in primary biliary cirrhosis (PBC) (Graham-Brown, Sarkany and Sherlock, 1982; Powell, Rogers and Dickson, 1983) and in chronic active hepatitis (CAH) (Rebora *et al*, 1978; Rebora, Rongioletti and Canepa, 1982; Rebora and Rongioletti, 1984). Recently, it was reported that there was a high prevalence of hepatitis C virus (HCV) infection in patients with LP. The first report documented in 1991 suggested a possible relation between HCV infection and LP (Mokni *et al*, 1991), and it might explain some aspects of the association between LP and CLD. The prevalence of anti-HCV antibodies in patients with cutaneous lichen planus (CLP) and/or oral lichen planus (OLP) ranged from 3.8 to 65% (Divano, Parodi and Rebora, 1992; Rebora *et al*, 1992; Bagan *et al*, 1994; Cribier *et al*, 1994; Gandolfo *et al*, 1994; Nagao *et al*, 1995; Tanei, Watanabe and Nishiyama, 1995; Carrozzo *et al*, 1996; Sanchez-Perez *et al*, 1996; Chosidow *et al*, 1997; Bagan *et al*, 1998; Dupond *et al*, 1998; Mignogna *et al*, 1998). The majority of the studies were conducted in countries where there were high prevalences of HCV infection in general populations, especially southern Europe and Japan. However, the studies from the UK (Ingafou *et al*, 1998) and the Netherlands (van der Meij and van der Waal, 2000), where the prevalences of HCV infection was low, did not find any serological evidence of antibodies to HCV. Besides, two studies from Germany where the prevalence of HCV infection was also low found conflicting results: Imhof *et al* (1997) in a controlled study found a significant association between LP and HCV while Grote *et al* (1998) did not apparently find the association in an uncontrolled study. In the USA all but one of the performed studies reported a significant association between LP and HCV infection (Bellman, Reddy and Falanga, 1995; Chuang *et al*, 1999; Beaird *et al*, 2001; Chainani-Wu *et al*, 2001; Eisen,

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SEN virus infection in patients with chronic liver disease and hepatocellular carcinoma in Thailand

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Background. SEN virus (SENV) has been recently identified as a candidate agent of non-A-E hepatitis virus. However, the exact role of this novel virus in the pathogenesis of chronic liver disease, including chronic hepatitis and cirrhosis, and the development of hepatocellular carcinoma (HCC) remains to be established. ***Methods.*** Using seminested polymerase chain reaction (PCR) amplification to detect SENV-D and SENV-H strains in serum, we investigated SENV infection in voluntary blood donors and in patients with chronic liver disease and HCC. ***Results.*** SENV was detected in 5 of 100 blood donors (5%), in 15 of 60 patients with chronic liver disease (25%), and in 25 of 60 patients with HCC (42%). The prevalence of SENV in patients with HCC was higher than that in patients with chronic liver disease ($P = 0.05$) and in blood donors ($P < 0.001$). An age-specific prevalence of SENV was found at high levels among individuals aged 21–40 years, but was not detected among individuals in the lower age group. No differences between SENV-infected and non-infected patients were demonstrated with respect to demographic data, assumed source of infection, biochemical abnormalities, and severity of chronic liver disease and HCC. Moreover, SENV infection had no apparent effect on the survival of patients with HCC. ***Conclusions.*** Our data suggest that SENV infection is frequent among patients with chronic liver disease and HCC. However, pathogenic effects associated with SENV infection in chronic liver disease and HCC need further investigation.

Key words: hepatitis virus, SEN virus, chronic liver disease, hepatocellular carcinoma, Thailand

Introduction

Chronic liver disease (including chronic hepatitis and cirrhosis) and hepatocellular carcinoma (HCC) are common in Thailand and have been known to be associated with chronic hepatitis B virus (HBV) and chronic hepatitis C virus (HCV) infection.[1] Nonetheless, there is still a significant proportion of cases in which the etiology is unknown, which is suggestive of the existence of additional causative agents.[2] With the advent of sophisticated molecular biological techniques, two novel hepatitis virus candidates, designated hepatitis G virus (HGV) and TT virus (TTV) were recently identified, in 1995 and 1997, respectively.[3–5] Despite the worldwide distribution of HGV and TTV, the association of these viruses with liver disease has not clearly emerged. Most recent studies indicate that HGV and TTV are relatively harmless to the liver and are not the etiological agents in the majority of patients with cryptogenic chronic liver disease and HCC.[6–8]

SEN virus (SENV) represents the latest hepatitis virus, which was first isolated from the serum of an intravenous drug user (IVDU) infected with human immunodeficiency virus (HIV).[7,9] SENV was initially described as a single-stranded DNA virus of approximately 3600–3900 nucleotides and possessing at least three open reading frames.[7,9] Subsequent genomic and molecular evolutionary analyses have demonstrated that it is a small single-stranded circular virus that belongs to the superfamily of TTV-related viruses.[10] To date, eight distinct strains of SENV (A-H) have been identified. Among them, it has been shown that two SENV strains (SENV-D and SENV-H) are significantly associated with transfusion-associated non-A-E hepatitis.[11] SENV-D and SENV-H were also detected more frequently in patients with chronic liver disease and HCC than in healthy adults.[12,13] Despite its high prevalence among patients with liver disease, the exact role of SENV infection regarding the etiology of chronic liver

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Interspousal transmission of hepatitis C in Thailand

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Background. Previous studies evaluating the possibility of interspousal sexual transmission of hepatitis C virus (HCV) have yielded many conflicting results. Our study was carried out to determine the exact potential and risk factors of interspousal HCV transmission. ***Methods.*** The spouses (54 men and 106 women; mean age ±SD, 48 ± 8 years) of 160 patients with HCV infection (106 men and 54 women) were serologically tested for HCV using a third-generation enzyme-linked immunosorbent assay (ELISA). Positive results were confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). For positive couples, the cluster nucleotides of the HCV gene and genotypes were compared on the basis of restriction fragment length polymorphism (RFLP), Innogenetic Line Probe Assay (INNO-LiPA), and direct sequencing. Similarly, phylogenetic tree and sequence homology analysis was performed in order to precisely verify interspousal transmission. Risk factors promoting interspousal HCV transmission were also identified. ***Results.*** Throughout a mean duration of exposure of 23 + 5 years, most of the 160 partners had their usual and unprotected sexual relationships with the index patients. HCV-associated antibodies and HCV-RNA were detected in only 3 (1.88%) of the 160 spouses. Furthermore, homology and phylogenetic tree analysis could not clearly demonstrate that any one of these 3 positive spouses was infected with the same strain of HCV as that identified in the index cases. Because a positive group remained elusive, risk factors of interspousal HCV transmission could not be determined in this study. ***Conclusions.*** According to this study, interspousal transmission of HCV seems to be very rare. HCV-positive spouses should be firmly reassured that they can maintain their normal marital life.

Key words: hepatitis C virus, transmission, RFLP, risk factors

Introduction

Hepatitis C virus (HCV), the principal cause of non-A, non-B hepatitis,[1] is an RNA virus belonging to the family of *flaviviridae*; an estimated 170 million people, or about 3% of the global population, have been infected with HCV.[2] The virus can be grouped into at least six genotypes in different geographic areas.[3] Infection with different genotypes may affect the clinical outcome and response to treatment.[4–7]

Hepatitis C virus is transmitted by direct percutaneous exposure to infected blood products, such as the transfusion of various derivatives of blood products, and by intravenous drug abuse, which are well-established causes.[8,9]

To prove the mode of transmission, the identification of common strains of HCV can be performed by various methods, e.g., genotyping and polymorphism analysis,[10] direct sequencing of the genome, and phylogenetic analysis.[11]

Although sexual contact has been implicated as a route of transmission, the results are still controversial.[12–18] Infrequent sexual transmission of HCV has been shown in studies performed in Western countries, but many studies originating from Asia suggest that interspousal transmission may be crucial for the interfamilial spread of HCV, with a longer duration of marriage as the most evident risk factor.[17,18] Sexual transmission was documented in the presence of coexistent HIV infection, and this suggested the co-transmission of HCV and HIV to be more efficient than HCV transmission alone.[19]

To determine more accurately the epidemiology of interspousal HCV-transmission and possible risk

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Molecular epidemiology of gibbon hepatitis B virus transmission

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Although transmission of human hepatitis B virus (HBV) variants to nonhuman primates is well documented, it remains to be elucidated whether nonhuman primate HBV is transmissible to humans. The prevalence and transmission routes of gibbon HBV were analysed in 101 captive gibbons in Thailand. Approximately 40 % of these animals showed at least one marker of HBV infection; 19 animals were chronic HBV carriers, characterized by elevated levels of alanine amino transferase and the presence of HBV DNA. Some of the chronic animals were found to be anti-HBc (HBV core antigen) negative (4 of 19), while precore promoter point mutations (nt 1762 or 1764) were determined in four animals by RFLP analysis. Phylogenetic tree analysis of the complete surface gene sequences revealed that gibbon viruses clustered separately from hepadnaviruses of other hosts. Evidence for horizontal and vertical transmission in captive gibbons was obtained. HBV DNA was also detected in the saliva of HBV carrier gibbons. Although some of the animal caretakers at the Krabok Koo Wildlife Breeding Centre were found to be chronic HBV carriers, genotype and sequence analysis did not reveal any evidence for zoonotic disease transmission.

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INTRODUCTION

Hepatitis B virus (HBV), a small double-shelled virus that contains a partially double-stranded DNA genome of approximately 3200 bases (White & Fenner, 1994), is found in several species, including woodchuck, ground squirrel, a range of bird species, such as duck, goose and grey heron (Mason *et al.*, 1980; Marion *et al.*, 1980; Summers *et al.*, 1978), and nonhuman primates like chimpanzee (*Pan troglodytes*), woolly monkey (*Lagothrix lagothrica*), orang-utan (*Pongo pygmaeus*) and gorilla (*Gorilla gorilla*) (Grethe *et al.*, 2000; Lanford *et al.*, 1998; Vaudin *et al.*, 1988; Warren *et al.*, 1999). HBV was also isolated from a gibbon, infected in the wild and housed at the CDC for 2 years (Mimms *et al.*, 1993). Phylogenetic analysis of the complete genome revealed that gibbon HBV represents a unique group when compared to HBV genotypes described previously. Remarkably, a 33 bp deletion after the start codon of preS1, the most divergent part of the genome, was observed (Norder *et al.*, 1996). Nonrecognition of an anti-preS1 monoclonal antibody at aa 27–35 to gibbon virus particles confirmed that the gibbon HBV surface protein conformation is different from that of human HBV (Mimms *et al.*, 1993).

Phylogenetically, HBV isolated from gibbons and chimpanzees share an early lineage, indicating that these viruses were indigenous to their respective hosts (Norder *et al.*, 1996). On the other hand, infection of chimpanzees with human and gibbon HBV can be accomplished (Gallagher *et al.*, 1991). Experimental transmission of human HBV to gibbons by exposure to human saliva containing HBV has been reported also (Bancroft *et al.*, 1977; Scott *et al.*, 1980). Replication of human HBV in the respective animals supported the close relation of these hosts and may indicate natural HBV cross-transmission. On the other hand, no evidence has been obtained thus far for HBV transmission from gibbon or chimpanzee to human. HBV is present at levels as high as 1×10^{13} virions ml^{-1} in the blood of HBV e antigen (HBeAg)-positive patients but virus particles have also been found in other body fluids, including saliva/nasopharyngeal fluids, semen, cervical secretions and

The GenBank accession numbers of the sequences reported in this paper are AF274495–96, AF274499, AF275378, AF477482–94, AY077735–36 and AF529308–09.

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Human Herpesvirus Infection in Children with Fever and Maculopapular Rash

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SUMMARY Fever with maculopapular rash is a common problem in children. Infection with human herpesviruses is one of the common etiologies in fever with rash. The aim of this study has been to examine patients presenting with fever and maculopapular rash without respiratory symptoms for human herpesviruses infection by using multiplex nested-polymerase chain reaction. A descriptive and prospective study was conducted at King Chulalongkorn Memorial Hospital, Bangkok, Thailand from June 2000 to December 2001. One hundred patients, 43 boys and 57 girls, aged between 2 months and 14 years were recruited. Human herpesvirus 6 (HHV6) was the most common (24%) whereas HHV7, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) were present in 9%, 3% and 2% of the patients, respectively. Four percent of the patients simultaneously harbored HHV6 and HHV7. Only one patient had CMV, HHV6 and HHV7. Patients with HHV7 had a mean age of 4.5 years, whereas those with HHV6 had a mean age of 1.6 years. HHV6 and HHV7 were commonly found as causes of fever and maculopapular rash without respiratory symptoms. Co-infection with different herpesviruses can be found in the same patient.

Fever with maculopapular rash without a localized sign is a common problem in children. It may be due to viral infection, bacterial infection, or drug allergy.[1,2] Viral infections commonly causing maculopapular rash are attributed to cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus (HHV)-6, HHV-7, enterovirus and parvovirus B-19.[1-6]

Human herpesviruses (HHVs) can cause primary infection, which may proceed towards latency.[7,8] They are responsible for acute febrile illnesses in children. Viral reactivation in immunocompromised patients has been linked to a number of diseases and causes significant morbidity and mortality, especially in transplant recipients and HIV patients.[9,11]

Serologic evidence, such as a four-fold or greater increase of the IgG titer, does not differentiate between primary or reactivated latent infection.[12-14] The detection of HHV6 and HHV7 in peripheral blood leucocytes (PBL) only is of limited significance since viral DNA persists in the PBL of healthy persons. Viral DNA detection in a cell free body fluid such as plasma has been shown to correlate with active viral replication.[15,16] Plasma polymerase chain reaction has demonstrated diagnostic accuracy in detecting primary HHV6 infection in immunocompetent children with a sensitivity and specificity amounting to 90% and 100%, respectively.[12,17] Polymerase chain reaction (PCR) has become one of the most widely used techniques in human virology diagnostic. Multiplex nested-PCR represents a modification of the original PCR protocol applied to simultaneously amplify DNA originating from different pathogens using several primer pairs in the same reaction.[18] Based on a technique described previously,[18] we applied a sensitive multiplex nested-PCR to determine the prevalence of human lymphotropic herpesviruses in children with fever and maculopapular rash.

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DESIGN OF DEGENERATE PRIMERS FOR MULTIPLEX NESTED-PCR DETECTION OF HUMAN LYMPHOTROPIC HERPESVIRUSES

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Abstract. To develop the rapid diagnosis and typing of human lymphotropic herpesviruses by using multiplex nested-PCR, the primary PCR (1° PCR) primers were redesigned as degenerate primers based on a highly conserved sequences of each DNA polymerase gene of EBV, CMV, HHV-6, HHV-7 and HHV-8. The forward degenerate primer (HHV/1+) contained 12 different sequences, whereas there were 8 different sequences in the reverse degenerate primer (HHV/1-). Optimization of multiplex nested-PCR assay conditions were performed to search for the appropriate amount of degenerate primers, dNTP, *Taq* DNA polymerase, template of secondary PCR (2° PCR) and annealing temperature used in 1° PCR reaction. Detection sensitivity was the same as described in previous report (approximately 10-100 genome copies). To ensure a true negative result, PCR detection of hepatitis B virus genome was used as internal control. Our presented results, the designed degenerate primers could be used to detect various types of HHV by multiplex nested-PCR.

INTRODUCTION

Epstein-Barr virus (EBV, HHV-4), human cytomegalovirus (CMV, HHV-5), human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7) and human herpesvirus 8 (HHV-8, Kaposi's sarcoma associated herpesvirus) are known human lymphotropic herpesvisus (HHVs) whose natural host is human. Although the viruses are different from one another, regarding their biological behavior and genomic arrangements, they share the same ability to establish latency after primary infection (Roizmann *et al*, 1992). Recurrent or reactivated HHVs infection are commonly found as opportunistic diseases in HIV-infected person (Fabio *et al*, 1997; Schulz, 1998; Clark, 2000) or in immunosuppressed patients following bone marrow, kidney, liver, or heart transplantation (Chan *et al*, 1997; Osman *et al*, 1997; Clark, 2000). HHV-6 and HHV-7 have also been associated with febrile illness and childhood diseases, exanthem subitum (roseola infantum) (Yamanishi *et al*, 1988; Tanaka *et al*, 1994). Nevertheless, EBV appear to be an important etiological factor for nasopharyngeal carcinoma. The use of serum/plasma EBV DNA as a reliable tumor marker prior to, during, and after treatment of the cancer was reported (Liebowitz *et al*, 1994; Shotelersuk *et al*, 2000).

Recently, PCR-based assays have been recognized as sensitive and specific method for molecular detection and identification of HHVs (Wakefield *et al*, 1992; Tenorio *et al*, 1993; Vandevanter *et al*, 1996; Clark *et al*, 1997; Kidd *et al*, 1998; Minjolle *et al*, 1999; Pozo and Tenorio, 1999; Johnson *et al*, 2000; Kessler *et al*, 2000; Kearns *et al*, 2001). A multiplex nested-PCR for simultaneous detection and typing of HHV4 (EBV), HHV-5 (CMV), HHV-6, HHV-7 and HHV-8 was developed by Francisco Pozo and Antonio Tenorio in 1999. Two sets of specific primers, designed for amplification of a highly con-

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Relationship between vasoactive intestinal peptide and intrapulmonary vascular dilatation in children with various liver diseases

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Aim: To evaluate the potential of vasoactive intestinal peptide (VIP) as a pathogenic factor of intrapulmonary vascular dilatation (IVD) in hepatopulmonary syndrome (HPS). *Background:* HPS comprises a triad comprising liver dysfunction, IVD and hypoxaemia. Although the pathogenesis of the process has not been elucidated, many vasodilating substances, such as VIP, have been implicated in the development of pulmonary vascular abnormalities. IVD can be detected by contrast-enhanced echocardiography (CEE) before the development of abnormal gas exchange. *Methods:* Forty-two children (20M, 22F; mean age 4.39 ± 4.17 y) with various liver diseases who attended the paediatric liver clinic of King Chulalongkorn Memorial Hospital between March 2000 and February 2001 were recruited to the study. Each patient was tested for transcutaneous O_2 saturation, CEE (applying the agitated normal saline technique), liver function test and serum VIP level. *Results:* Fourteen of the 42 patients (33%) were CEE positive. Only one of the 14 patients had associated hypoxia and clinical cyanosis. The serum VIP levels of children with liver disease were significantly higher than those of the controls (60.21 ± 35.04 pg/ml vs 43.71 ± 34.61 pg/ml, $p = 0.03$). CEE-positive children tended to have higher serum VIP levels than CEE-negative children (72.65 ± 40.31 vs 53.99 ± 31 pg/ml, $p = 0.3$). The serum VIP levels of biliary atresia (BA) patients with favourable outcomes (serum bilirubin ≤34 μmol/L) were not significantly different from those with unfavourable outcomes (serum bilirubin >34 μmol/L) (42.95 ± 14.53 pg/ml vs 66.07 ± 32.17 pg/ml, $p = 0.5$).

Conclusions: CEE is a non-invasive test for early detection of IVD in children with liver disease. VIP is not solely responsible for the pathogenesis of IVD in HPS. Further studies are required to determine which substances cause the development of IVD.

Key words: *Intrapulmonary vascular dilatation, liver disease, vasoactive intestinal peptide*

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The pathogenesis of hepatopulmonary syndrome (HPS), a clinical triad comprising chronic liver disorder, pulmonary gas exchange abnormalities (PaO_2 < 70 mmHg or an alveolar-arterial oxygen gradient >20 mmHg), and intrapulmonary vascular dilatation (IVD), without intrinsic cardiopulmonary disease, has as not yet been elucidated despite numerous investigations (1). Increased production and secretion of vasodilators by the liver, or impaired hepatic clearance and metabolism of circulating vasodilatory substances or factors inducing them may alter pulmonary vascular blood flow. There is scant evidence to implicate decreased levels of vasoconstricting factors such as serotonin or endothelin in HPS.

Vasoactive intestinal peptide (VIP) is a neurotransmitter as well as a potent vasodilator (2, 3). Infusion of VIP into the pulmonary artery of animals causes marked pulmonary vasodilatation (4, 5). Iwabuchi et al. proposed that VIP caused pulmonary vasodilatation, which is mediated via an endothelium-derived relaxing factor or nitric oxide (6). Reduction in the pulmonary vascular resistance index occurred after infusion of VIP into the peripheral veins of normal human subjects (7). Elevated plasma VIP levels in cirrhotic patients are mainly caused by impaired hepatic clearance of VIP, rather than portosystemic shunting (8, 9). So far, there has not been any direct evidence to prove that VIP causes abnormality of the pulmonary circulation in HPS.

No correlation has been shown between either the severity of portal hypertension or liver dysfunction, with clinical manifestation and pulmonary functional outcomes of HPS. HPS is generally associated with the relatively common symptoms and signs of hypoxaemia observed in patients with chronic liver disease, although it often manifests itself subclinically. Therefore, it is advisable to perform various tests aimed at diagnosing

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Molecular characterization of hepatitis-A-virus infections, in the context of two outbreaks in southern Thailand

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As hepatitis A virus (HAV) is usually transmitted through the faecal–oral route, hepatitis A is a communicable disease. In countries of intermediate to low endemicity, sudden outbreaks of human infection with the virus may occur. Between September 2001 and April 2002, there were two outbreaks of HAV infection in the Ruso and Yeengor districts of Narathiwas province, in southern Thailand. Isolates of HAV were recovered during these outbreaks, from 14 in-patients with acute hepatitis in Ruso (12 positive for anti-HAV IgM and all positive for HAV RNA), 16 children with asymptomatic infection in Yeengor (14 positive for anti-HAV IgM and nine for HAV RNA), and four isolated cases in Bangkok (all positive for anti-HAV IgM). Molecular characterization of the VP1–P2A region of each isolate was followed by phylogenetic analysis. All of the isolates from Narathiwas province were found to be of genotype 1a, to have the same VP1 nucleotide sequence, and to show a high level of sequence homology (≥99.5%) with the isolates from Bangkok and with previous Thai isolates. These results should facilitate further research into HAV transmission and genotype identification in community outbreaks.

The hepatitis A virus (HAV) was once placed in the genus *Enterovirus*, within the picornavirus family (Melnick, 1982), but has recently been reclassified as a prototype genus of the new genus, *Hepatovirus* (Minor, 1991). The HAV genome can be divided into three functional regions: P1, P2 and P3 (Rueckert and Wimmer, 1984). The P1 region encodes the capsid polypeptides VP1, VP2 and VP3 and perhaps the putative VP4. The P2 and P3 regions encode the non-structural protein which is necessary for virus replication. The virion is a non-enveloped, 27-nm particle, with 7.5 kb of positive-sense RNA.

The VP1–P2a region is now usually used to classify the genotype. There are at least seven genotypes that have been described world-wide (Robertson *et al.*, 1991, 1992; Taylor, 1997). Viruses from genotypes I, II, III and VII have been recovered from cases of hepatitis A in humans, whereas types IV, V and VI have only been isolated from simian species. Most human HAV isolates belong to genotypes I and III, with 80% of them belonging to genotype I. Genotypes I and III are further divided into subtypes a and b but II and VII are represented by only one human strain each (Robertson *et al.*, 1992). Subgenotype Ia is the predominant genotype in North America whereas Ib appears to predominate in Europe and the Mediterranean region (Robertson *et al.*, 1992). In general, human HAV isolates of diverse epidemiological origin appear to be closely related (Robertson *et al.*, 1992), although the level of VP1–P2a-sequence relatedness might be correlated with the geographical origin of the viruses (Jansen *et al.*,

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Detection and Differentiation of Human Herpesviruses 1-5 by Consensus Primer PCR and RFLP

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SUMMARY **Eight human viruses of the *Herpesviridae* family represent a significant public health problem world-wide. Detection and typing of five of the human herpesviruses (HSV-1, HSV-2, VZV, EBV, and CMV) was performed by applying a consensus primer polymerase chain reaction (PCR). The amplified PCR products from the five human herpesviruses were typed based on their restriction enzyme digestion polymorphism with *Hinf* I and *Alu* I. Fifteen clinically suspected specimens from herpesvirus-infected patients were also evaluated. A fragment of the DNA polymerase gene from each of the five human herpesviruses was successfully amplified by the set of consensus primers. Their amplicons obtained by PCR from the template DNAs were subjected to restriction endonuclease digestion and human herpesviruses 1-5 could be clearly differentiated and typed. This method can be used to detect and differentiate between the five human herpesviruses in clinical specimens. This study demonstrates the value of testing for five human herpesviruses by consensus PCR and restricted fragment length polymorphism (RFLP). These procedures are simple and straightforward techniques for the investigation of clinical specimens.**

Eight human viruses of the *Herpesviridae* family- herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesviruses 6, 7 and 8 (HHV-6, 7 and 8) - are a significant public health problem world-wide. These viruses have been classified as members of the alpha-herpesvirus subgroup (HSV-1, HSV-2 and VZV), beta-herpesvirus subgroup (CMV, HHV-6 and HHV-7) and gamma-herpesvirus subgroup (EBV and HHV-8).[1,2] Whereas some of the agents have been known for decades and are well characterized, little is known about the pathogenic potentials of some of the more recently described members of this viral family.

Before the introduction of molecular techniques, laboratory diagnosis of viral infections relied on cell culture for virus isolation, detection of specific antibody or detection of viral antigen. The clinical signs produced by alpha-herpesviruses (HSV-1, HSV-2 and VZV) are fever with vesicular lesions. In normal hosts, it is easy to differentiate the clinical presentations of HSV and VZV. In immunocompromised hosts, however, skin lesions are not typical. Tzanck smear and skin biopsy can not differentiate between HSV and VZV.[3] Serological diagnosis is of value only to determine past exposure. VZV is a highly contagious virus that needs isolation and requires higher doses of antiviral therapy. CMV and EBV manifest as fever with maculopapular rashes. They have clinical importance in immunocompromised-hosts because all human herpesvi-

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Comparative study of different methods to genotype hepatitis C virus type 6 variants

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Abstract

Hepatitis C virus (HCV) genotype 6a is found frequently in Southeast Asia. In Thailand, however, genotype 6 variants may exist which posses a genotype 1 like sequence in the 5′ non-coding region. In order to genotype correctly these viruses, four different methods; INNO-LiPA assay, two RFLP assays on the core region (using different restriction enzymes) and phylogenetic analysis of the core sequences were compared. Samples from 17 chronic HCV patients from the Netherlands and Thailand and 18 anti-HCV positive blood donors recruited from Thailand were tested. The INNO-LiPA could not distinguish genotype 6 variants. The RFLP methods used could not, or only in combination with 5′NCR genotyping methods, identify type 6 variants. In conclusion, for identification of type 6 variants at least two different regions of the HCV genome have to be analyzed (both 5′NCR and core).

Keywords: HCV; Genotyping; RFLP; INNO-LiPA; Sequencing

1. Introduction

Hepatitis C virus (HCV), a positive-stranded RNA virus of approximately 9400 nucleotides, has been shown to be a major etiologic agent of parentally transmitted non-A, non-B hepatitis (Choo et al., 1989). Chronic hepatitis develops in approximately in 80% of all HCV infections, whereas cirrhosis develops eventually in 20% of these patients (Alter et al., 1992). Hepatocellular carcinoma, with and without cirrhosis, has also been associated with HCV infection (De Mitri et al., 1995).

Sequence comparisons of viral variants from different geographical areas have led to the identification and classification of at least six major genotypes, many of which contain a number of more closely related, yet distinct subtypes of the virus (Simmonds et al., 1993a; Stuyver et al., 1994). Phylogenetic analysis of complete genomic sequences (Okamoto et al., 1992) or even relatively short subgenomic regions such as core, E1, NS4 or NS5, may be used for virus classification into genotypes. The overall sequence similarities over the complete genome are at least 91% between variants of the same genotype, approximately 79% between subtypes, and about 68% between different genotypes (Tokita et al., 1994).

It has been postulated that differences in nucleotide sequence could result in differential activity of HCV proteins that could alter the rate of HCV replication, sensitivity to the antiviral activity of interferon, or pathogenicity of the virus (Simmonds, 1995). In recent years, substantial evidence has emerged indicating that typing and subtyping for HCV is important clinically; genotype 1 in particular, cannot be treated efficiently with IFN-α, while genotypes 2 and 3 respond favorably (Tsubota et al., 1994; Yoshioka et al., 1992). Moreover,

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Upregulation of IL-10 gene expression in porcine peripheral blood mononuclear cells by porcine reproductive and respiratory syndrome virus

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Several lines of evidence suggest that porcine reproductive and respiratory syndrome virus (PRRSV) may have immunomodulatory effects on the host immune system. To determine the effect of PRRSV on cytokine production, a multiplex PCR was established. This allowed a semi-quantitative analysis of IFN-γ, IL-2, IL-4, IL-10 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene expression levels from porcine peripheral blood mononuclear cells (PBMCs). These results showed that both live and inactivated PRRSV predominantly upregulated IL-10 gene expression in porcine PBMCs. In addition, when PBMCs from pigs immunized previously with classical swine fever virus (CSFV) vaccine were cultivated with the recall antigen, CSFV, in the presence of PRRSV, significant upregulation of IL-10 gene expression and reduction of IFN-γ gene expression were observed. These findings indicated that the presence of PRRSV in the culture could affect recall antigen response. This study implies that the induction of IL-10 production may be one of the strategies used by PRRSV to modulate host immune responses.

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INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is caused by PRRS virus (PRRSV), an enveloped, positive-stranded RNA virus that belongs to the family *Arteriviridae* (Snijder & Meulenberg, 1998). PRRSV has been recognized as one of the major aetiological agents of porcine respiratory disease complex, which causes a serious health problem in the pig industry worldwide (Halbur, 1998). Although the mechanism(s) by which PRRSV undertakes to invade the host immune system is unclear, several studies suggest that PRRSV may negatively modulate the host immune system (reviewed by Lager & Mengeling, 2000; Molitor *et al.*, 1996).

PRRSV is, phenotypically, a highly variable virus. It generally causes a persistent infection and induces a wide range of secondary infections (Wardley *et al.*, 1996). Following an infection, PRRSV persists in the infected pigs for up to 12 weeks and the infectious virus can be shed during this stage (Wills *et al.*, 1997). Although PRRSV is highly contagious, virus replication appears to be limited mainly to phagocytic cell populations, including macrophages and activated monocytes (Molitor *et al.*, 1996). However, proinflammatory cytokines were mostly undetectable or minimally increased following exposure to the virus (Van Reeth & Nauwynck, 2000). In most cases, there is a lack of correlation between the amount of viral antigen and the degree of pathological lesions, suggesting the possibility of immune-mediated pathogenesis rather than a direct effect of virus infection (reviewed by Lager & Mengeling, 2000).

Immune responses to PRRSV have been studied extensively and virus-specific cellular responses, including lymphocyte proliferation, delayed-type hypersensitivity, cytotoxic activity and cytokine production, have been demonstrated in PRRSV-infected pigs. However, there seemed to be a delay in the onset of these responses, as compared to other pathogens. Cellular immune responses to PRRSV are not usually detected until 4 weeks after PRRSV infection (Bautista & Molitor, 1997; Lopez Fuertes *et al.*, 1999). In contrast, the cellular immune response to other viruses, such as classical swine fever virus (CSFV), can be detected within a week following virus infection (Suradhat *et al.*, 2001). Although PRRSV induces a strong antibody response within the first week post-infection, neutralizing antibodies are not detected until the fourth week after infection, long after the virus is cleared from circulation (Yoon *et al.*, 1995). Thus, there appears to be a delay in the induction of cell-mediated and humoral immune responses in PRRSV-infected pigs.

Studies with regard to the role of porcine cytokines in immune regulation in pigs have been limited by the lack of porcine cytokine-specific immunological and biological assays. Recently, knowledge of the roles of cytokines in immunopathology and host–pathogen interactions has increased rapidly (Wood & Seow, 1996). RT-PCR has been shown to be a sensitive and effective method for

MOLECULAR CHARACTERIZATION OF HEPATITIS C VIRUS (HCV) CORE REGION IN HCV-INFECTED THAI BLOOD DONORS

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Summary. – In order to investigate the distribution of Hepatitis C virus (HCV) genotypes in Thailand, we performed phylogenetic analysis based on the virus core region and in this way we identified and reliably distinguished HCV genotypes 1–6 as well as subtypes. Among 100 plasma samples randomly selected from blood donors positive for antibodies to HCV (anti-HCV) 90 (90%) were found positive for HCV RNA and 77 of them were subjected to nucleotide sequencing. The following types and subtypes were identified in this group: 1a in 16 samples (20.8%), 1b in 14 samples (18.2%), 3a in 29 samples (37.7%), 3b in 5 samples (6.5%), and 6a in 13 samples (16.9%). Although this study allowed identification and characterization of HCV among blood donors, more extensive studies are needed to explore the HCV distribution in other population groups and in other geographical regions and to exploit the virus core-based characterization of HCV for evaluation of treatment and clinical outcome and epidemiological purposes.

Key words: HCV; virus core; blood donor; phylogenetic tree; genotype; subtype

Introduction

HCV infection, which can cause chronic liver diseases, cirrhosis and hepatocellular carcinoma, is still a major worldwide problem with approximately 170 million (3%) people already infected and 3–4 million de novo infected each year. (World Health Organization, 1997)

HCV, a positive single-stranded RNA virus of approximately 9.4 kb showing structural similarities with flaviviruses and pestiviruses, and hence closely related to Dengue, Japanese B encephalitis and Yellow fever viruses, has been identified as the major etiological agent of the parenterally transmitted non-A, non-B hepatitis (NANB, Choo *et al.*, 1989).

Upon comparing the sequences of its variants collected from different geographical areas, at least six major genotypes have been identified and classified. Among these, many contain a variety of more closely related but distinctive subtypes of the virus (Simmonds *et al.*, 1993). Attention has been focused on clinically relevant differences in liver disease caused by HCV, which may be attributable to infection with different genotypes, for example, leading to patients' different responses to the interferon therapy (Martinot-Peignoux *et al.*, 1998). Virus classification into genotypes may be achieved by phylogenetic analysis of complete genomic sequences (Okamoto *et al.*, 1992) or genomic regions such as core, E1, NS4 or NS5. Yet, as the respective genotypes are apparently crucial for patient management and as investigating the distribution of these sequences in Southeast Asia is imperative, sequences in the core region have been amplified which led to a reliable distinction of types 1–6 (Mellor *et al.*, 1996). The core region is relatively well conserved with nucleotide sequence similarity ranging from 81 to 88% in isolates of different genotypes (Simmond *et al.*, 1994). Due to the high degree of conservation these regions have been chosen for

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Abbreviations: HCV = Hepatitis C virus; NANB = non-A, non-B hepatitis; RFLP = restriction fragment length polymorphism; PCR = polymerase chain reaction; RT-PCR = reverse transcription – PCR; IVDU = intravenous drug user; LiPA = line probe assay

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SHORT COMMUNICATION

Human Metapneumovirus Infection in Thai Children

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Human metapneumovirus (hMPV) associated with clinical respiratory tract infection (RTI) in children was first isolated in the Netherlands. Of 120 Thai paediatric patients with RTI examined, 5 cases (4.2%) showed detectable hMPV based on N-gene-specific RT-PCR. All of them were negative for hRSV infection. Aligning the sequences with a reference strain revealed some nucleotide differences, which necessitates future investigation to evaluate clinical significance and genotype variation.

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INTRODUCTION

Respiratory tract infection (RTI) is a major public health problem that causes morbidity and mortality among children worldwide (1, 2). The major aetiological agents of RTI are viruses, e.g. human respiratory syncytial virus (hRSV), influenza A virus, influenza B virus, human parainfluenza virus type 1–3, adenovirus and rhinovirus (3). However, in only 60% of patients with RTI could the causative agent be identified (4). Recently, human metapneumovirus (hMPV), a new human virus first described in the Netherlands, has been isolated from children with severe respiratory tract disease (5, 6).

hMPV is a member of the Metapneumovirus genus within the *Pneumovirinae* subfamily, and belongs to the *Paramyxoviridae* family. Electron microscopy shows paramyxovirus-like pleiomorphic particles with short envelope projections. The nucleocapsid contains approximately 13.3 kb single-stranded, non-segmented RNA of negative polarity (5, 6). The genomic constellation is most closely related to avian pneumovirus (APV) serotype C, which causes upper respiratory tract disease in turkeys (5, 6). The clinical symptoms of hMPV are similar to those of hRSV infection, ranging from mild respiratory symptoms to severe cough, bronchiolitis and pneumonia (5–8). Serological surveys in the Netherlands have shown that all children over the age of 5 y had been exposed to hMPV. Moreover, the virus has been circulating in the human population for at least 50 y (5).

In 2002, hMPV was also found in North America (9), Australia (7, 8) and the UK (4), suggesting that it is likely to exist globally (5, 9). As commercially available diagnostic test kits for hMPV are not yet available, data on the prevalence of this virus are limited. This study was designed to investigate the prevalence of hMPV in nasopharyngeal secretions of infants and young children who presented with clinical symptoms of RTI, using the reverse-transcription polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Population study

120 nasopharyngeal secretions were collected from paediatric patients with clinical symptoms of RTI. None of them was immunocompromised or undergoing immunosuppressive therapy. The patients had either attended the outpatient clinic or been admitted to the paediatric ward, Chulalongkorn Hospital, Bangkok, in March 2001 to September 2002. The specimens were collected and stored at −70°C until further testing.

Human metapneumovirus detection

RNA extraction was performed according to the guanidine method (10). The RNA pellet was resuspended in 10 µl diethylpyrocarbonate-treated sterile water (Dep-C water) and directly used as a template for complementary DNA (cDNA) synthesis. The RNA template was added to 20 µl cDNA reaction mixture containing 250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT, 10 mM dNTP, 20 U RNase inhibitor (Promega, WI, USA) and 25 U multireverse transcriptase (Promega), and incubated at 37°C for 1 h.

At the time of the study, 1 virus sequence originating from the Netherlands had been published in GenBank, on which the de novo primer design was based. Two pairs of oligonucleotide primers, 10 pmol each, specifically designed to amplify regions in the nucleoprotein (N) gene (GenBank accession no. AF371337) were used for nested PCR. The primer sequences were: 5′ ACG GGG TAG AGA AGA GCT GG 3′ [nucleotides (nt) 389–408] for the outer forward primer MPVP F, 5′ GCA AAG TTG GGA CAG TTG GC 3′ (nt. 985–1004) for the outer reverse primer MPVP R, 5′ GCA TCA ACC ATA GAA GTG GGA C 3′ (nt. 556–577) for the inner forward primer MPVN F, and GCA TTG TTT GAC CGG CCC CA 3′ (nt. 795–814) for the inner reverse primer MPVN R.

The first PCR amplification round was performed in a total volume of 50 µl containing both outer forward and reverse primers, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 2 U *Taq* polymerase (Finnzymes, CA, USA) and 10 mM dNTP. The PCR conditions comprised 1 initial denaturation cycle at 94°C for 1 min, followed by 35 cycles at 94°C for 1 min (denaturation), 54°C for 1 min (primer annealing), 72°C for 1 min (extension) and a final extension step at 72°C for 7 min. The first round PCR product was further amplified using the inner sense and anti-sense primers. Otherwise, the conditions were identical to those applied in the first round. The second round PCR products were separated by

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CASE REPORT

Varicella Infection in a Pediatric AIDS Patient Presenting as Umbilicated Papules

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SUMMARY An 8-year-old girl with acquired immunodeficiency syndrome presented with fever and alteration of consciousness. She had a history of persistent cryptococcal meningitis. She developed multiple discrete umbilicated papules that resembled cutaneous cryptococcosis on the second day of admission. Skin biopsy revealed an ulcer with a wedge-shaped necrosis of the dermis. The edge of the ulcer showed intracellular edema, margination of nucleoplasm and multinucleated cells, consistent with herpes infection. The diagnosis of varicella-zoster virus infection was confirmed by the identification of herpesvirus DNA from the lesion and differentiation from other herpesviruses by restriction fragment length polymorphism (RFLP) method. Intravenous acyclovir was given at a dose of 500 mg/m^2, three times daily for 14 days which resulted in resolution of the skin lesions within 2 weeks.

Varicella (chickenpox) is caused by the varicella-zoster virus (VZV). It occurs most often in children younger than 10 years of age. In normal children, its systemic symptoms are usually mild; serious complications are extremely rare. Immunocompromised patients, with either primary or recurrent (zoster) infection, are at increased risk of severe disease.[1,2] In human immunodeficiency virus infection, during profound immunosuppression, the primary infection with varicella-zoster virus may manifest as an atypical form. We report varicella in a girl, with acquired immunodeficiency syndrome, presenting as umbilicated papules. Only 4 cases of varicella-zoster virus infection presenting as umbilicated papules have been reported.[3,4] The diagnosis of varicella-zoster virus infection was confirmed by detection of herpesvirus DNA from the lesion and differentiation from other herpesviruses by restriction fragment length polymorphism (RFLP) method.[5,6]

CASE REPORT

An 8-year-old girl had vertical transmission of HIV infection. She received d4T and ddI for 2 years, but had to discontinue the medication 4 months prior to admission due to severe bone marrow suppression, confirmed by bone marrow aspiration. Three months before this admission, she was admitted to the hospital due to cryptococcal meningitis, and was treated with intravenous amphotericin B for 78 days, which resulted in some clinical improvement. However, she still had persistent cryptococcal antigen in her cerebrospinal fluid. During

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SEN Virus Infection and the Risk of Hepatocellular Carcinoma: A Case-Control Study

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OBJECTIVE: Hepatitis B virus (HBV) and hepatitis C virus (HCV) are major risk factors for hepatocellular carcinoma (HCC). The role of a novel DNA virus, designated SEN virus (SENV), in the etiology of liver cancer remains to be established. The aim of this study was to evaluate the association between SENV infection and the risk of HCC by conducting a hospital-based, case–control study among Thai patients.

METHODS: Eighty-six patients with HCC were enrolled and matched individually to a control according to sex, age (± 5 yr), and geographic background. The presences of HBV DNA, HCV RNA, and SENV DNA in stored serum samples were detected with the use of semi-nested polymerase chain reaction amplification.

RESULTS: Individuals who were infected with SENV did not have increased risk of developing HCC (OR = 1.49, 95% CI = 0.50–4.42). In contrast, those who were positive for HBV markers (hepatitis B surface antigen and/or HBV DNA) or HCV markers (anti-HCV and/or HCV RNA) had significant risk for HCC (OR = 19.91, 95% CI = 8.26–47.98 and OR = 7.97, 95% CI = 2.15–29.54, respectively). Moreover, coinfection with SENV did not further increase the risk of HCC among patients infected with HBV and/or HCV.

CONCLUSION: Our data suggest that, unlike chronic HBV or HCV infection, SENV infection is not a risk factor for developing HCC in Thai populations. (Am J Gastroenterol 2003;98:2500–2504. © 2003 by Am. Coll. of Gastroenterology)

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, particularly in sub-Saharan Africa and Southeast Asia, where its prevalence is high (1). In Thailand, HCC is the most common malignant tumor, with an incidence of 6.8 per 100,000 in men and 2.3 per 100,000 in women per year (2). Various risk factors have been associated with the development of HCC, such as dietary exposure to aflatoxin, heavy alcohol consumption, and chronic infection with hepatitis viruses, in particular hepatitis B virus (HBV) and hepatitis C virus (HCV) (3). Epidemiological studies indicate that the relative roles of these two viruses in hepatic carcinogenesis vary considerably among different populations. In China and Southeast Asia, for examples, HBV is considered a primary risk factor for HCC. Conversely, HCV plays an important role in the development of liver cancer in Japan and Europe (4). However, there are a significant number of HCC cases for which the etiology remains unknown, suggestive of the existence of additional causative agents.

The recently discovered SEN virus (SENV) is a single-stranded DNA virus of approximately 3600–3900 nucleotides that belongs to the *Circoviridae* family (5, 6). So far, eight distinct genotypes of SENV (A–H), which share 40–50% sequence homology, have been identified. Among them, it has been shown that two SENV genotypes (SENV-D and SENV-H) are significantly associated with transfusion-associated non-A–E hepatitis (7). Also, these genotypes were more frequently detected in patients with chronic liver disease and HCC than in healthy adults (8, 9). Despite its high prevalence among patients with liver disease and HCC, the exact role of SENV infection in the etiology of liver cancer remains unclear. In fact, most information on SENV prevalence and its clinical association comes from case series, in which their confounding effects from selection bias could not be excluded. However, case–control studies on the association between SENV infection and the risk of HCC have never been performed.

The aim of the current study was, therefore, to evaluate the association between SENV infection and the risk of HCC by conducting a hospital-based, case–control study among Thai patients. We also assessed the interactive roles of this novel virus together with HBV and/or HCV infections in HCC causation.

MATERIALS AND METHODS

Patients

Stored serum samples obtained from 86 patients with diagnosed HCC who were admitted to the Department of Internal Medicine, Chulalongkorn University, Bangkok, Thailand, between March, 1998 and September, 1999 were used

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Serum Hepatocyte Growth Factor and Clinical Outcome in Biliary Atresia

By Paisarn Vejchapipat, Apiradee Theamboonlers, Rapeepan Chaokhonchai, Voranush Chongsrisawat, Soottiporn Chittmittrapap, and Yong Poovorawan
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Purpose: Biliary atresia (BA) remains one of the most intractable liver diseases leading to liver fibrosis. Serum hepatocyte growth factor (HGF) has been shown to increase in cirrhotic patients. The aim of this study was to investigate the possible role of HGF in BA.

Methods: Serum levels of HGF were determined using an enzyme-linked immunosorbent assay from 28 BA patients and 25 healthy children. The patients were categorized into 3 groups according to their clinical outcomes (good, fair, and poor): group A (good), jaundice-free patients (TB < 2.0 mg%); group B (fair), patients with mild to moderate jaundice (TB, 2 to 10 mg%); and group C (poor), patients with marked jaundice (TB > 10 mg%). Unpaired *t* test and analysis of variance (ANOVA) with post-hoc tests were used. Data were expressed as mean and SEM.

Results: Serum HGF levels in BA patients were higher than the controls ($P = .02$). Subgroup analysis found that there were 12 patients in group A, 8 patients in group B, and 8 patients in group C. The mean age of patients in groups A, B, and C were 5.34 ± 0.52, 7.45 ± 1.98, and 5.49 ± 1.57 years ($P > .05$). Serum HGF in controls and groups A, B, and C were 0.24 ± 0.03, 0.28 ± 0.04, 0.36 ± 0.09, and 0.56 ± 0.07 ng/mL, respectively. Serum HGF levels in BA patients with poor outcome were higher than patients with good outcome ($P = .02$). There was no difference in serum HGF of BA patients with fair outcome compared with other groups.

Conclusions: Serum HGF is elevated in BA. Furthermore, BA patients with poor outcome have significantly elevated HGF compared with patients with good outcome. Serum HGF levels may be predictive of prognosis with respect to the progression of liver dysfunction. However, the results of HGF in patients with fair outcome are inconclusive, probably because of the small sample size. Further studies are needed to elucidate the detailed mechanisms.

INDEX WORDS: Biliary atresia, hepatocyte growth factor.

AQ: 1

BILIARY ATRESIA (BA), the absence of patent extrahepatic bile ducts, still remains one of the most intractable liver diseases in children. When patients with BA are left without any surgical management, the majority will die of hepatic decompensation, esophageal variceal bleeding, or infection.[1,2] Currently, it is accepted that hepatic portoenterostomy or Kasai operation at the early age is indispensable to the successful management of infants with BA,[3] especially in countries in which a liver transplantation program is not widely established. A wide spectrum of the results of Kasai operation has been published. According to a report from Japan, of more than 900 patients who underwent the surgical correction, 59% of patients were jaundice free, 21% had their jaundice decreased, whereas in 20% jaundice persisted.[4] Hence, despite Kasai operation, a number of patients with BA finally have end-stage cirrhosis caused by progressive hepatic fibrosis.[1,3,4] However, the exact cause of the fibrosis or cirrhosis in BA patients is still unclear.

During the last 15 years, hepatocyte growth factor (HGF) has been identified as the most potent mitogen for primary hepatocytes.[5] The effects of HGF are mediated by a specific receptor encoded by the c-*met* proto-oncogene.[6] HGF has subsequently been shown to be a multifunctional cytokine. The functional properties of HGF include regeneration, antifibrosis, and cytoprotection.[7] Recently, HGF has been clarified to have an antifibrogenic property in dimethylnitrosamine-induced hepatic fibrosis in rats.[8] It has been shown that serum HGF levels increased in patients with damaged liver[9] including hepatic resection[10] and liver tumors after chemotherapy.[11]

Currently, there is little information available regarding the role of HGF in the pathophysiology of BA. Because progressive hepatic fibrosis is an important development in patients with BA together with the possible role of HGF in fibrogenesis, there may be some links between the pathophysiology of BA and the levels of serum HGF. The aims of the study were to evaluate the serum HGF levels in BA patients and to investigate

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SHORT COMMUNICATION

Seroprevalence of Cytomegalovirus Infection in Children Born to HIV-1 Infected Women

Sasithorn Likitnukul[1], Parvapan Bhattarakosol[2] and Yong Poovorawan[1]

SUMMARY Cytomegalovirus (CMV) is a frequent opportunistic infectious agent in children infected with human immunodeficiency virus type 1 (HIV-1). It has been implicated as a factor in the progression of HIV-1 disease. The aim of the present study was to evaluate the prevalence of CMV infection in Thai children born to HIV-1 infected women. The prevalence of CMV infection was 13, 89 and 84% in HIV-infected children and 9, 61 and 75% in HIV uninfected at age ranges of 0-12, 13-36 and 37-79 months, respectively. The prevalence of CMV infection was significantly different between HIV infected children (89%) and HIV uninfected (61%) at the age of 13-36 months ($p < 0.05$). The presence of CMV IgM in some children of age < 1 year suggested that CMV infection could occur early in life. Early co-infection may be important as they remain a risk factor for reactivation of latent CMV infection throughout the course of the HIV diseases. Clinical monitoring and appropriate work up may be of benefit in the early diagnosis and treatment of CMV disease.

Cytomegalovirus (CMV) is a DNA virus and member of the *Herpesviridae* family. The virus is ubiquitous and commonly infects persons of all ages worldwide. The prevalence of the infection, which increases with age, is higher in developing countries especially among the lower socioeconomic population than in the more developed ones. A study on the prevalence of CMV antibodies revealed a seroconversion rate of 83.7% in Thai children below the age of sixteen years, and 70.7% to 100% in adult blood donors and pregnant women.[1-5] Most CMV infections are inapparent, but this virus can also cause a number of clinical illnesses that vary in severity from mild to fatal. In normal immunocompetent persons, the infection is occasionally characterized by a mononucleosis-like syndrome. Among immunosuppressed individuals, including recipients of transplants and patients with acquired immunodeficiency syndrome (AIDS), a variety of clinical symptoms may manifest during primary CMV infection or reactivation. Pneumonia, retinitis and gastrointestinal diseases are common and can be fatal.[6] CMV-infection has been implicated as a cofactor in the progression of HIV-1 disease. Our objective was to determine the prevalence of CMV infection in Thai children born to HIV-1 infected women.

MATERIALS AND METHODS

Patient population

One hundred and sixty children born to HIV-1 infected women who were receiving health care services at Chulalongkorn Memorial Hospital between October 1993-July 1998 were studied. The patients were recruited after informed consent was obtained from their parents. After reviewing their clinical status and the diagnosis of HIV infection, these children were categorized into HIV-infected children, and

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The Effect of Phenobarbital on the Accuracy of Technetium-99m Diisopropyl Iminodiacetic Acid Hepatobiliary Scintigraphy in Differentiating Biliary Atresia from Neonatal Hepatitis Syndrome

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Abstract

Biliary atresia (BA) and neonatal hepatitis syndrome (NHS) are major causes of cholestatic jaundice in infancy. Technetium-99m diisopropyl iminodiacetic acid hepatobiliary scintigraphy (^{99m}Tc-DISIDA scan) is widely used in the differentiation of these two entities. The objective of this study was to evaluate the effect of phenobarbital premedication on the accuracy of ^{99m}Tc-DISIDA scan. Ninety-five cholestatic infants (38 females and 57 males) with an age range of 2 weeks to 4 months (mean 2.1 mo) who underwent ^{99m}Tc-DISIDA scan testing were retrospectively reviewed. The patients were divided into 3 groups according to the history of phenobarbital administration prior to ^{99m}Tc-DISIDA scan examination. Group 1 (n = 48), group 2 (n = 29), and group 3 (n = 18) received phenobarbital at the dosage of 5 mg/kg/day for at least 5 days, less than 5 mg/kg/day or less than 5 days, and no premedication, respectively. The accuracy of ^{99m}Tc-DISIDA scan in differentiating BA from NHS in group 1, 2, and 3 was 72.92 per cent, 89.66 per cent, and 100 per cent, respectively. No significant difference was seen between the patients who received and did not receive phenobarbital in terms of age at presentation, age at onset of jaundice, and liver function tests. In conclusion, phenobarbital therapy may not be necessary prior to ^{99m}Tc-DISIDA scan examination in the evaluation of cholestatic infants and thus a delay in diagnosis and surgical therapy of BA can be avoided.

Key word : Scintigraphy, Phenobarbital, Biliary Atresia, Neonatal Hepatitis

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Orangutan herpesvirus

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Abstract: A male orangutan suffered from ulcers at the buccal mucosa. We obtained swab fluid from the base of both vesicles and ulcers and collected blood for further separation into serum, plasma and peripheral blood mononuclear cells (PBMC) for detection of antibody to herpesvirus by serology and herpesvirus DNA by polymerase chain reaction (PCR) using consensus degenerate primers. Serology was positive for human EBV IgG but negative for Epstein–Barr virus (EBV) immunoglobulin (IgM), as well as for both human cytomegalovirus and herpes simplex virus IgG and IgM. Upon PCR, we obtained a 232-bp product of virus DNA from PBMC, but not from lesions, serum or plasma. We confirmed the positive result by direct sequencing and compared the nucleotide sequence with other nucleotide sequences applying the BLAST program from GenBank. The sequence was similar to lymphocryptovirus of macaque (93%), marmoset (93%), gorilla (90%) and human EBV (90%). We aligned this sequence with other sequences in GenBank and performed phylogenetic analysis, showing that it probably belongs to the gammaherpesvirus group.

Key words: apes – EBV – herpesvirus – LCV – monkey – PCR – serology

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Introduction

Epstein–Barr virus (EBV) is a gammaherpesvirus associated with malignant diseases in humans. EBV is associated with the development of Burkitt's lymphomas, B cell lymphomas in immunosuppressed hosts, nasopharyngeal carcinoma, Hodgkin's disease and gastric carcinomas. EBV infection is typically initiated by transmission via oral secretions, virus replication in the oropharynx, and infection of peripheral blood B lymphocytes [10]. Thereafter, EBV persists as a latent infection in a small fraction of B lymphocytes and replicates sporadically at low levels in the oropharynx. Latent EBV infection is generally asymptomatic but can lead to lymphoproliferations and lymphomas when individuals are immunosuppressed, either due to congenital immunodeficiencies, medication received after transplantation or human immunodeficiency virus infection [18].

Most Old World non-human primates, such as macaques, baboons, chimpanzees and apes, are infected with herpesviruses of the same subgroup [lymphocryptovirus (LCV)] as EBV [1, 5] and could provide a model for EBV infection. Like humans, almost all Old World primates have been naturally infected with their endogenous LCV by adulthood, have LCV-infected B cells in the peripheral blood and maintain serum antibody responses for life [6]. These antibodies cross-react with EBV antigens and can be used to identify LCV infection in non-human primates [3, 8, 11].

Epstein–Barr virus-related herpesviruses or LCV genera are known to naturally infect both Old and

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Serum Hyaluronan: A Marker of Liver Fibrosis in Patients with Chronic Liver Disease

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SUMMARY The aim of this study was to evaluate the clinical significance of serum hyaluronan (HA) as a marker of liver fibrosis in patients with chronic liver disease. Serum HA was measured by an ELISA-based method in 28 patients with chronic hepatitis (CH), 43 patients with liver cirrhosis (LC), 57 patients with hepatocellular carcinoma (HCC) and 60 healthy controls. Mean serum HA concentration in patients with LC was 1,376.80 ± 2,568.85 ng/ml which was significantly higher than those in patients with CH, HCC and the controls (575.93 ± 732.58, and 426.36 ± 687.33, and 117.86 ± 311.11 ng/ml, respectively). Based on a ROC curve analysis, a cut-off point of 354 ng/ml discriminated between LC and other groups with a sensitivity, specificity and accuracy of 82.4%, 78.2%, and 80.2%, respectively. Mean HA concentrations were correlated with the degree of liver fibrosis, but not the grade of necroinflammatory activity. In patients with LC, the mean serum HA level was significantly increased in the Child C group (3,977.96 ± 4,906.21 ng/ml) in comparison with the Child B and A groups (1,002.63 ± 448.55, and 537.90 ± 424.16 ng/ml, respectively). We conclude that serum HA concentrations reflect the extent of liver fibrosis and severity of cirrhosis. Thus, serum HA can be a diagnostic marker of liver fibrosis and cirrhosis in patients with chronic liver disease.

Chronic liver disease of any etiology may lead to the development of liver fibrosis and cirrhosis. Liver fibrosis is characterized by increased deposition and altered composition of extracellular matrix (ECM) components in the portal tracts, around central veins or in perisinusoidal spaces.[1] The progresssive accumulation of ECM distorts the liver architecture and consequently compromises hepatocyte function, causing life-threatening complications such as variceal bleeding, ascites and liver failure. Currently, liver biopsy remains the standard method of assessment of liver fibrosis and cirrhosis. However, the use of liver biopsy in clinical practice has several limitations, such as hemorrhage, discomfort, sampling error and the cost of hospitalization. Therefore, several biochemical techniques have been studied as surrogate makers of liver fibrosis, which would obviate or greatly reduce the need of liver biopsy. These biochemical markers include products of collagen synthesis or degradation (e.g. type III procollagen peptide, type IV collagen 7S domain), and serum levels of enzymes involved in matrix turnover (e.g. tissue inhibitor of metalloproteinases). The ECM glycoproteins and proteoglycan/glycosaminoglycan such as fibronectin and hyaluronan have also been examined.[2,3]

Hyaluronan (hyaluronic acid, HA), ubiquitously distributed in the extracellular spaces, is a linear

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• LIVER CANCER •

Role of serum total sialic acid in differentiating cholangiocarcinoma from hepatocellular carcinoma

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Abstract

AIM: This study was designed to evaluate the clinical application of serum total sialic acid (TSA) in the diagnosis of cholangiocarcinoma (CCA).

METHODS: Serum TSA was determined by periodate-resorcinol microassay in 69 patients with CCA, 59 patients with hepatocellular carcinoma (HCC), 37 patients with cirrhosis, 61 patients with chronic hepatitis and 50 healthy blood donors.

RESULTS: The mean serum TSA concentration in CCA (2.41±0.70 mmol/L) was significantly higher than those of HCC, cirrhosis, chronic hepatitis and healthy blood donors (1.41±0.37 mmol/L, 1.13±0.31 mmol/L, 1.16±0.26 mmol/L, and 1.10±0.14 mmol/L, respectively; $P<0.001$). Based on ROC curve analysis, a cut-off point of 1.75 mmol/L discriminated between CCA and HCC with a sensitivity, specificity and accuracy of 82.6 %, 83.1 %, and 82.8 %, respectively.

CONCLUSION: Based on our results, serum TSA would be a useful marker for the differential diagnosis of CCA from HCC.

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INTRODUCTION

Cholangiocarcinoma (CCA) constitutes a common primary liver cancer in Southeast Asia where the liver fluke, *Opisthorchis viverrini*, is endemic[1]. Most patients with CCA are diagnosed at advanced stages, therefore, treatment of the cancer is usually palliative and the prognosis is poor[2]. Currently, there is no 'gold standard' tumor marker for the diagnosis of CCA. This is particularly remarkable for early detection of the tumor itself, for screening of the high-risk groups, and for differentiating CCA from hepatocellular carcinoma (HCC), another primary liver cancer which is common in Southeast Asia and frequently associated with chronic hepatitis B or C[3]. Among the available serum tumor markers, the most commonly used is a high-molecular-weight glycolipid, carbohydrate antigen 19-9 (CA 19-9). CA 19-9, however, is not a sensitive or specific tumor marker for CCA. As a single diagnostic test, CA 19-9 increases in approximately 65 % of liver fluke-associated CCA[4]. Elevated concentrations of this marker have also been observed in patients with a variety of gastrointestinal cancers, as well as benign cholestasis and acute cholangitis[5]. As a result, a more sensitive and specific serum marker for the diagnosis of CCA is considered necessary.

Sialic acid, a class of important ketoses that contain nine carbon atoms, is an acetylated derivative of neuraminic acid (2-keto-5-amino-3, 5-dideoxy-D-nonulosonic acid)[6]. The unique structural features of this molecule, which includes a negative charge owing to a carboxyl group, enable it to play an important role in cellular functions, such as cell-to-cell recognition and transformation to malignancy[7]. Elevated levels of serum total sialic acid (TSA) have been reported in patients diagnosed with various cancers such as lymphoma, malignant melanoma, lung cancer and gastrointestinal cancers[8,9]. Recently, it has been shown that most patients with CCA have an elevated concentration of serum TSA, and determination of this marker yields high diagnostic values that differentiate between CCA and benign hepatobiliary diseases[10]. However, the diagnostic role of the serum marker in discriminating CCA from HCC has never been verified.

Therefore, the aim of this study was to use a simple technique (microassay) to determine the clinical application of serum TSA in the diagnosis of CCA by comparison with HCC and other chronic liver diseases including chronic hepatitis and cirrhosis.

MATERIALS AND METHODS

Subjects

Sera for the measurement of TSA levels were obtained from 5 groups of subjects who were attending King Chulalongkorn Memorial Hospital and Udonthani Hospital from January 1998 to July 1999.

Group 1 consisted of 50 adult healthy blood donors as control subjects.

Group 2 consisted of 61 patients with chronic hepatitis which was diagnosed based on histopathology.

Group 3 consisted of 37 patients with cirrhosis. The diagnosis of cirrhosis was based on histopathology and/or clinical features such as the presence of ascites, or esophageal varices.

Group 4 consisted of 59 patients with HCC. The diagnosis of HCC was based on histopathology and/or imaging techniques combined with serum alpha-fetoprotein levels above 400 ng/ml.

Group 5 comprised 69 patients with CCA. All patients in this group were residents of Thailand's northeastern provinces where *O. viverrini* was endemic. The peripheral type CCA was diagnosed based on liver tumor features detected by ultrasound/CT scan and confirmed by histology. Criteria for

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• CASE REPORT •

Unusual manifestations of gastric inflammatory fibroid polyp in a child

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Abstract

AIM: Inflammatory fibroid polyp (IFP) is a rare benign lesion that may occur throughout the digestive tract. IFP is more commonly found in the antrum of the stomach in particular. It mostly affects adults at the average age of 60 years. These polyps are able to cause abdominal pain, gastrointestinal bleeding, intestinal obstruction or intussusception. In this paper we report a case of gastric IFP with unusual presenting features.

METHODS: A child with gastric IFP was described and the literature was reviewed.

RESULTS: A 4-year-old girl presented with fever for 2 months, arthralgia of knees and ankles, iron deficiency anemia, and hypoalbuminemia. Her stool examination was positive for occult blood. The upper gastrointestinal study demonstrated a large lobulated mass at the upper part of gastric body. Partial gastrectomy *en bloc* with this 5cm×8 cm mass was subsequently performed. Pathological examination was consistent with IFP. Following the mass excision, her fever abruptly declined and disappeared together with anemia and arthralgia. She remained asymptomatic and the abdominal ultrasonography performed at the 24-month follow-up demonstrated no recurrence of the tumor.

CONCLUSION: The etiopathogenesis of IFP still remains unclear. The presence of IFP throughout the gastrointestinal tract and its variable clinical appearances make it difficult to diagnose. The inflammatory symptoms found in this patient support the hypothesis of inflammatory benign lesions of IFP.

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INTRODUCTION

Gastric neoplasms are exceedingly rare in children. Murphy *et al.* reviewed 1 403 pediatric gastric pathology reports and found only 3 benign gastric tumors[1]. Attard *et al.* reported hyperplastic-inflammatory polyp was the most common gastric polyps (42%) found in pediatric population[2].

Inflammatory fibroid polyp (IFP) is a solitary polypoid or sessile lesion with an inflammatory basis. It is a rare benign lesion that may occur throughout the digestive tract, but is most often seen in the stomach (approximately 80%)[3]. IFP in the stomach is usually located in the antrum or prepyloric region[4,5]. In large retrospective studies of gastric polyps, 3.1-4.5% were found to be IFP[6,7]. It is slightly more common in women (female:male ratio 1.6:1)[5]. It is found in all age groups, although not often in children, and its maximal incidence is in the sixth decade[7]. The symptomatology is determined by its site. In the stomach, it causes pyloric obstruction, and often in the small bowel, intussusception which is the most common presentation in children.

We report a case of gastric IFP who presented with prolonged fever, arthralgia, hypoalbuminemia, and iron deficiency anemia. Surgical excision led to a complete resolution of those symptoms.

CASE REPORT

A 4-year-old girl presented with high fever for 2 months, arthralgia of knees and ankles, and anemia that required multiple packed red cell transfusions. The lowest hemoglobin was 3.9 g/dL. A provisional diagnosis of juvenile rheumatoid arthritis was made. She was treated with aspirin and prednisolone, but had no improvement. Later she was referred to our hospital in June 2001.

On her admission, the patient' s weight was 15 kg (25^{th} percentile for gender and age). Physical examination revealed pale conjunctivae, pitting edema of both legs, the rest was unremarkable. Laboratory tests after multiple blood transfusions showed a white blood cell count of 41 400/mm^3, 76% PMNs, 16% lymphocytes, 6% monocytes, and 2% atypical lymphocytes; hemoglobin was 11.4 g/dL (MCV 76 fL, MCH 21.1 pg, MCHC 27.9 g/dL, RDW 21.2%), and platelet count was 684 000/mm^3. Stool occult blood was positive. Serum albumin and globulin were 2.1 g/dL and 3.2 g/dL, respectively. Anti DNA, ANA, ANCA, anti Smith, rheumatoid factor, VDRL and LE cell were negative. β_1C was 176.9 mg/dL. ESR was 82 mm/h. Culture of blood, urine, stool, and bone marrow aspirate were negative. Upper GI series demonstrated a large lobulated mass at the upper part of gastric body (Figure 1).

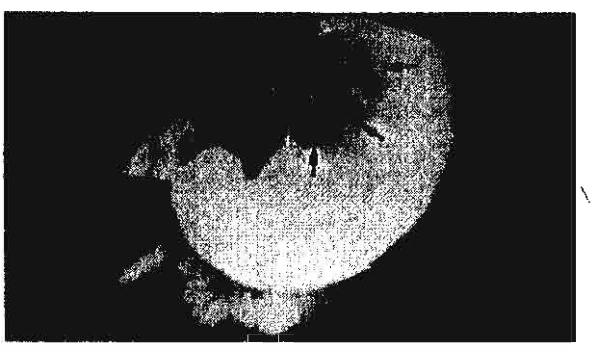

Figure 1 A large lobulated mass at the upper part of gastric body (arrow head) shown by radiography.

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Clinical features and molecular characterization of hepatitis A virus outbreak in a child care center in Thailand

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Abstract

Background: As a result of declining hepatitis A endemicity in Thailand, an increasing number of children and adolescents have become susceptible to hepatitis A virus (HAV) infection. *Objective:* The present study was aimed at both investigating the clinical features and determining molecular characterization of HAV during an outbreak, which occurred in a childcare center located in a suburban area of Bangkok between November 2002 and February 2003. *Methods:* Serum samples obtained from all children in the center were tested for anti-HAV IgG and anti-HAV IgM. Testing for HAV–RNA was performed in sera, saliva and stool samples by the reverse transcription-polymerase chain reaction (RT-PCR) with primers located at the VP1-2A region. To further characterize the HAV genotype serum derived HAV–RNA-positive PCR products were sequenced. *Results:* Anti-HAV IgG and anti-HAV IgM were detected in 74 and 70 of 112 children in the center, respectively. Among those positive for anti-HAV IgM, 65 cases were asymptomatic, while five children had acute clinical hepatitis. The ratio between symptomatic and asymptomatic children was 1:13. Among the asymptomatic cases, 31 (47.7%) displayed biochemical hepatitis with elevated alanine aminotransferase (ALT) levels. All the isolates from this outbreak were found to be of subgenotype IA, which showed a high level of sequence homology with previous Thai isolates. HAV–RNA could not be detected in saliva, but was found in stool for at least 3 weeks after initial diagnosis of clinical or biochemical hepatitis. *Conclusion:* Because of the infection's characteristically asymptomatic spread, hepatitis A poses an increased risk to childcare centers. The presence of a single sub-genotype indicates that this HAV strain has been predominantly circulating in Thailand.

Keywords: Hepatitis A; HAV; Outbreak; Childcare center

1. Introduction

Hepatitis A virus (HAV) infection has been a major public health problem in many developing countries worldwide (Cuthbert, 2001). In Thailand during the previous two decades, HAV infection has undergone a remarkable regression from high to intermediate endemic levels. This shifting epidemiology of hepatitis A has been attributed to general improvements in hygiene, living standards and socioeconomic progress (Poovorawan et al., 2002). As a result, the proportion of children and adolescents susceptible to the infection has increased and major outbreaks caused by contaminated water and food have been periodically reported (Poovorawan et al., 2000). For instance in 1992, an outbreak caused by contaminated drinking water occurred among schoolchildren in a province in southern Thailand (Sinlaparatsamee et al., 1995). Between September 2001 and April 2002, two more recent major community outbreaks occurred in another province in southern Thailand (Theamboonlers et al., 2002).

HAV is a 7.5 kb positive-stranded RNA virus belonging to the *Picornaviridae* family (Yokosuka, 2000). The virion comprises three functional regions namely, P1, P2 and P3. The P1 region encodes the structural proteins VP1, VP2, VP3, and putative VP4, while the P2 and P3 regions encode nonstructural proteins associated with viral replication

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Simultaneous quantitation and genotyping of hepatitis B virus by real-time PCR and melting curve analysis

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Abstract

Hepatitis B virus (HBV) genotype and HBV DNA levels have been implicated in clinical evaluation and prognosis of patients with chronic HBV infection. The aim of the present study was to develop a rapid and sensitive method for simultaneous HBV DNA quantitation and differentiation between HBV genotypes B and C in a single-step reaction by real-time PCR and melting curve analysis using SYBR Green I fluorescent dye. The genotypes obtained by this method were compared with those examined by PCR-RFLP and direct sequencing on 52 serum samples of patients with chronic HBV infection. Using the results obtained by direct sequencing and phylogenetic analysis as the reference, the accuracy of HBV genotyping by PCR-RFLP and melting curve analysis was 90.38 and 92.31%, respectively. The geometric mean of HBV DNA levels was 3.42×10^6, 2.10×10^6, 1.19×10^5 and 3.10×10^4 copies/μl in asymptomatic carriers, patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma, respectively. It is concluded that this method has the advantages of rapidity, reproducibility and accuracy, which would be feasible and attractive for large-scale analysis, particularly in regions where HBV genotypes B and C are prevalent.

Keywords: Real-time PCR; Melting analysis; Genotypes; HBV

1. Introduction

Hepatitis B virus (HBV) infection is a public heath problem worldwide with an estimated 350 million people are infected chronically with the virus (Maddrey, 2000). Chronic HBV infection is associated with a diverse clinical spectrum, ranging from asymptomatic carrier status over chronic hepatitis and cirrhosis to hepatocellular carcinoma (HCC). HBV, a member of the hepadnaviridae, has a relaxed-circular, partially double stranded DNA genome of approximately 3200 nucleotides, and has been classified into eight genotypes (A–H) based on an inter-group divergence of more than 8% in the entire genomic sequence (Kidd-Ljunggren et al., 2002; Arauz-Ruiz et al., 2002). It has been shown that most HBV genotypes have distinct geographical distributions. For instance, genotypes A and D are predominant in Western countries and India, whereas genotypes B and C prevail in Southeast Asia, China and Japan. Genotype E is restricted to Africa and genotype F is found in Central and South America.

Besides the differences in geographical distribution, there is growing evidence that the viral genotypes may influence the clinical outcomes of chronic HBV infection. In particular, among Asian patients who constitute approximately 75% of HBV carriers worldwide, it has been shown that HBV genotype C is associated with more severe liver diseases (Lindh et al., 1999; Kao et al., 2000a; Orito et al., 2001a; Chan et al., 2003), and it also has a lower response rate to antiviral treatment than genotype B (Kao et al., 2000b; Wai et al., 2002). These data suggest that the determination of HBV genotypes, especially genotypes B and C may constitute an essential part of the clinical evaluation in patients with chronic HBV infection. Currently, HBV genotyping is performed mainly by restriction fragment length polymorphism (RFLP) (Lindh

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