บทวิจารณ์

EBV เกี่ยวข้องอย่างมากกับการเกิดมะเร็งหลังโพรงจมูก ในการศึกษาครั้งนี้พบลักษณะทางพยาธิวิทยาของชิ้นเนื้อตามกำหนดขององค์การอนามัยโลกทั้ง 3 ชนิด แต่พบชนิดที่ 1 (WHO type1) จำนวนน้อยที่สุดคือประมาณ 5% ซึ่งคล้ายกับการศึกษาอื่น ๆ

การวินิจฉัยมะเร็งหลังโพรงจมูกทางห้องปฏิบัติการตั้งแต่แรกจนถึงปัจจุบันนิยมใช้วิธีทางซีโรโลยีอย่างไรก็ดีเมื่อเร็วๆ นี้มีรายงานการวินิจฉัยมะเร็งหลังโพรงจมูกด้วยวิธีการตรวจยีโนมของเชื้อด้วยเทคนิคทาง PCR การศึกษาครั้งนี้สามารถตรวจพบ EBV DNA ในชิ้นเนื้อของผู้ป่วยมะเร็งหลังโพรงจมูกจำนวน 92.9% คือ 156 รายจาก 168 ราย ขณะที่พบในกลุ่มควบคุมสูงถึง 62.5% คือ 15 รายจาก 24 ราย โดยที่ EBV ของทั้งสองกลุ่มส่วนใหญ่เป็น type A การพบ EBV DNA ในกลุ่มควบคุมสูงนี้ตรงกับที่มีรายงานในการศึกษาอื่น ๆ ด้วย เช่น พบว่า 70%-80% ของคนปกติ มี EBV DNA ใน nasopharyngeal tissue นอกจากนี้ 56% ของคนปกติมี EBV DNA ใน oropharyngeal cells ผลการศึกษาครั้งนี้ร่วมกับผลจากการศึกษาอื่น ๆ ล้วนให้การสนับสนุนแนวคิดที่ว่า แม้ว่า EBV จะเกี่ยวข้องอย่างมากกับการเกิดมะเร็งหลังโพรงจมูก แต่ปัจจัยอื่น เช่น สภาพแวดล้อม, ปัจจัยทางพันธุกรรม ก็น่าจะนำมาพิจารณาว่าเกี่ยวข้องกับการเกิดมะเร็งหลังโพรงจมูกด้วย

EBV พบได้ในคนทั่วไป oropharyngeal epithelial cells บริเวณคอเป็นแหล่งเพาะไวรัสที่ดี ชิ้นเนื้อ nasopharyngeal ของกลุ่มควบคุมอาจมีการติดเชื้อจากเชื้อ EBV ที่ถูกปล่อยออกมาในปากและคอ ดังนั้นจึงไม่ควรใช้การตรวจยีโนมของเชื้อด้วยเทคนิคทาง PCR เพื่อวินิจฉัยมะเร็งหลังโพรงจมูก

ในทางตรงข้ามการศึกษาครั้งนี้แสดงให้เห็นว่า ระดับแอนติบอดีชนิด IgG-, IgA-anti VCA ในซีรั่มผู้ป่วยมะเร็งหลังโพรงจมูกสูงกว่าในกลุ่มควบคุมอย่างมีนัยสำคัญดังที่เคยรายงานมาแล้ว อย่างไรก็ดี specific IgG-anti VCA สามารถพบในกลุ่มควบคุมในระดับสูงด้วย แม้ว่าไตเตอร์ $\geq$ 640 จะเกี่ยวข้องกับการเป็นมะเร็งหลังโพรงจมูกแต่ค่า specificity มีเพียง 58.3% ขณะที่พบ specific IgA-anti VCA ใน 89.7 % ของผู้ป่วยมะเร็งหลังโพรงจมูก และ 16.7% ของกลุ่มควบคุม ดังนั้น specific IgA-anti VCA จึงเหมาะสำหรับใช้วินิจฉัยมะเร็งหลังโพรงจมูก

บางรายงานจากประเทศจีนพบว่า ระดับของ IgA- หรือ IgG-anti EA จะลดลงเมื่อก้อนมะเร็งยุบหลังได้รับรังสีรักษา แต่ระดับแอนติบอดีจะกลับสูงขึ้นมาใหม่ในช่วง 6-8 เดือนก่อนที่ผู้ป่วยจะกลับเป็นใหม่ (recurrence) ขณะที่บางรายงานพบว่าระดับ IgA-anti VCA ลดลงในผู้ป่วยหลังรักษาหาย ขณะที่ระดับที่สูงของแอนติบอดีนี้ยังพบอยู่ในผู้ป่วยที่พบการกลับเป็นใหม่ของมะเร็ง จากการศึกษาครั้งนี้พบว่าในผู้ป่วยมะเร็งหลังโพรงจมูกไทยนั้น แอนติบอดีที่น่าจะใช้เป็นตัวบ่งชี้การตอบสนองต่อการรักษาน่าจะดู

ได้จากการลดลงอย่างน้อย 4 เท่าของระดับแอนติบอดีตัวใดตัวหนึ่งใน 3 ตัวนี้คือ แอนติบอดีชนิด IgG-anti VCA หรือ IgA-anti VCA หรือ IgG-anti EA อย่างน้อยหนึ่งชนิดโดยไม่พบการเพิ่มขึ้นของแอนติบอดีชนิดอื่นในขณะเดียวกันเลย หลักเกณฑ์นี้มีความแม่นยำ 74 % นอกจากนี้ยังสามารถใช้ลักษณะการลดลงของแอนติบอดีนี้ในทางอ้อมเพื่อเป็นตัวบ่งชี้ถึงการกลับเป็นใหม่ของมะเร็ง เนื่องจากไม่พบการลดลงของแอนติบอดีลักษณะดังกล่าวในผู้ป่วยกลุ่มนี้เลย

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Output

1. Papers

1.1 Specific IgA antibody to Epstein-Barr viral capsid antigen: the better marker for screening nasopharyngeal carcinoma than EBV-DNA detection by polymerase chain reaction. *Asian Pac J Allergy Immunol (in press)*

1.2 Determination of antibody marker(s) against Epstein-Barr virus antigen(s) as the indicator for responsive treatment and recurrence of nasopharyngeal carcinoma in NPC patients in Thailand. *Manuscript in preparation*

2. International Symposium

Kantakamalakul W, Chongkolwatana C, Naksawat P, *et al.* Specific IgA antibody to Epstein-Barr viral capsid antigen: a better marker for screening nasopharyngeal carcinoma than EBV-DNA detection by polymerase chain reaction. The 5th Asian Research Symposium in Rhinology, the Imperial Queen's Park Hotel, Bangkok, Thailand. January 27-28, 2000.

*หลังการประชุม คณะกรรมการจัดการประชุมดังกล่าวได้ทำจดหมายเชิญ (ดังเอกสารที่แนบมา) ให้ส่งเรื่องเพื่อตีพิมพ์เป็น Proceedings ของวารสาร Rhinology *(manuscript 2)*

3. การพัฒนาเทคนิคการ treat เซลล์ NC-37 ให้ express EA

ปัจจุบัน commercial kit สำหรับตรวจหาแอนติบอดีต่อ EA มีราคาสูงมาก ทุนวิจัยที่ได้รับไม่สามารถรองรับค่าใช้จ่ายตรงจุดนี้ได้ คณะผู้วิจัยจึงได้ทำการพัฒนาเทคนิคการ treat เซลล์ NC-37 ให้ express EA ขึ้น โดยติดต่อขอคำแนะนำจาก Prof. Annika Linde ที่สถาบัน Karolinska ประเทศสวีเดน รวมทั้งหัวหน้าโครงการได้แวะชมห้องปฏิบัติการดังกล่าวขณะไปประชุม WHO-UNAIDS Workshop ที่ Microbiology and Tumorbiology Center (MTC) ณ สถาบันดังกล่าว ขณะนี้เทคนิคดังกล่าวได้ถูกพัฒนาขึ้นจนสามารถใช้ได้แล้ว

หลังจากเสร็จสิ้นโครงการวิจัยนี้แล้ว คณะผู้ร่วมวิจัยเห็นพ้องต้องกันว่าทางภาควิชาจุลชีววิทยาจะเปิดบริการการตรวจหาแอนติบอดีชนิด IgG-, IgA-anti EA ขึ้น เนื่องจากสามารถเตรียมแอนติเจนดังกล่าวได้เองแล้ว ซึ่งราคาค่าใช้จ่ายจะถูกกว่าการสั่งซื้อน้ำยาจากต่างประเทศมาก และแพทย์สามารถใช้แอนติบอดีดังกล่าวร่วมกับ IgG-, IgA-anti VCA ซึ่งเปิดบริการอยู่แล้ว เพื่อเป็นตัวบ่งชี้ถึงการตอบสนองต่อการรักษาหรือการกลับเป็นใหม่ของผู้ป่วยมะเร็งหลังโพรงจมูกโดยจะเสียค่าใช้จ่ายน้อยลงมาก ซึ่งจะก่อประโยชน์ในการรักษาและติดตามผู้ป่วยอย่างยิ่ง

ASIAN RESEARCH SYMPOSIUM IN RHINOLOGY
(5th ARSR)

January 27-28, 2000 Bangkok, Thailand

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4 สิงหาคม 2543

เรื่อง ขอให้ส่งต้นฉบับเพื่อตีพิมพ์ใน Proceedings

เรียน ผู้ช่วยศาสตราจารย์แพทย์หญิงวรรณี กัณฐกมาลากุล

ตามที่ท่านได้เสนอผลงานในการประชุม the 5th Asian Research Symposium in Rhinology ซึ่งได้จัดที่โรงแรมอิมพีเรียลควีนส์ปาร์ค เมื่อวันที่ 27-28 มกราคม พ.ศ. 2543 ความตามทราบแล้วนั้น

คณะกรรมการจัดการประชุมดังกล่าว ได้ทำการคัดเลือกเรื่องที่น่าสนใจ เพื่อรวบรวมจัดพิมพ์เป็น Proceedings ของการประชุม โดยจะจัดพิมพ์เป็น supplement ของวารสาร Rhinology ซึ่งเป็น Official journal of the International Rhinologic Society. เป็นวารสารระดับนานาชาติทางสาขาโรคจมูก

เรื่องที่ท่านนำเสนอ คือ เรื่อง

1. Specific IgA antibody to epstein-barr viral capsid antigen : the better marker for screening nasopharyngeal cacinoma than ebv-dna detection by polymerase chain reaction.

ได้รับการคัดเลือกให้ตีพิมพ์ใน Proceedings นี้ด้วย จึงใคร่ขอเชิญให้ส่งต้นฉบับเรื่องนี้ในรูปแบบดังนี้

1. มีความยาวไม่เกิน 3 หน้ากระดาษ A4 (เป็น extended abstract)
2. มี reference ไม่เกิน 10 เรื่อง
3. รูปแบบของการเขียนต้นฉบับ และ การอ้าง reference ใช้ตามแบบที่กำหนดโดยวารสาร rhinology ตามเอกสารที่แนบมา
4. กำหนดส่งต้นฉบับภายในวันที่ 15 กันยายน 2543

คณะกรรมการจัดการประชุมได้มอบหมายให้ดิฉัน และ Associate Professor Ruby Pawankar ทำหน้าที่บรรณาธิการของ Proceedings นี้ และให้ดำเนินการจัดพิมพ์ให้แล้วเสร็จก่อนการประชุม the 6th Asian Research Symposium ซึ่งจะจัดที่ประเทศฮ่องกงในวันที่ 10-11 มีนาคม 2544 จึงใคร่ขอความร่วมมือให้จัดส่งต้นฉบับตามกำหนดด้วย จะขอบคุณยิ่ง

ขอแสดงความนับถือ

(ศาสตราจารย์แพทย์หญิงฉวีวรรณ บุนนาค)

ป.ล. หากเรื่องของท่านได้รับการตีพิมพ์ในวารสารอื่นแล้ว และไม่ประสงค์จะตีพิมพ์ซ้ำ ขอความกรุณาแจ้งให้ทราบด้วย และหากจะส่ง reprint มาให้ 1 ชุด ด้วยจะเป็นพระคุณยิ่ง

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ภาคผนวก

Manuscript 1

Specific IgA Antibody to Epstein-Barr Viral Capsid Antigen : A Better Marker for Screening Nasopharyngeal Carcinoma than EBV-DNA Detection by Polymerase Chain Reaction

a short running head: IgA anti EBV-VCA for screening NPC

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SUMMARY

Nasopharyngeal carcinoma (NPC) is strongly associated with Epstein-Barr virus (EBV) infection. To assess whether EBV DNA detection by polymerase chain reaction (PCR) or presence of specific serum antibody to viral capsid antigen (VCA) was a better marker for screening NPC, nasopharyngeal tissues and blood samples from 58 NPC patients and 24 non NPC patients (23 with laryngotracheal stenosis and 1 with chronic tonsillitis) were tested for the presence of EBV DNA and serum specific VCA antibodies, respectively. EBV DNA was detected in 56 (96.5%) of NPC patients and 15 (62.5%) of non-NPC controls, with predominant EBV type A in both groups. On the other hand, specific VCA IgA antibody was detected in the majority of NPC patients: 52 (89.7%) while only 4 (16.7%) were detected in non-NPC controls. Therefore, specific VCA IgA antibody may serve as a better marker for screening NPC than EBV DNA detected by PCR.

Key word: nasopharyngeal carcinoma (NPC); Epstein-Barr virus (EBV); specific viral capsid antigen (VCA) IgA-antibody; polymerase chain reaction (PCR)

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is one of the most common cancers in Southern China and South-East Asia including Thailand.[1-4] Epstein-Barr virus (EBV) has been found to be closely associated with this cancer. Pathmanathan *et al*.[5] showed new evidence confirming that EBV is the primary etiological agent in the development of NPC by demonstrating dysplastic lesions and carcinoma *in situ* in premalignant clones of EBV-infected cells. There are two different types of EBV, types A and B (or types 1 and 2). Although both types have been found in nasopharyngeal tissues, type A is more common.[6,7]

The presence of serum antibodies to EBV antigens have been used for the diagnosis of NPC.[8-11] IgG- and IgA-anti viral capsid antigen (VCA) antibody titers in NPC patients were found to be higher than that of healthy cases.[12-14] In addition, IgA-anti VCA was useful for the early detection of NPC in a field survey in China.[14] However, 15-20% of NPC patients were not identified using this antibody.[15,16] Other specific antibodies to EBV antigens have also been shown to be useful for the early detection of NPC.[17-19] So far, no single serological marker has been identified to be used as a screening assay for all NPC patients. Meanwhile, detection of EBV DNA in tissue by polymerase chain reaction (PCR) has recently been used as a supplement to the serologic screening of NPC[20] and as a screening method for NPC in a high risk group.[21,22] However, EBV DNA detected by PCR technique was also found in oropharyngeal cells, nasopharyngeal tissues and exfoliated cells from the postnasal space of a high number of healthy individuals.[23-25] The purpose of this study was to evaluate whether specific anti-VCA antibody or EBV DNA detection by PCR was a better marker for screening patients with NPC.

MATERIALS AND METHODS

Subjects and clinical specimens

Fifty-eight histologically confirmed NPC patients and 24 non-NPC controls who attended the Department of Otolaryngology, Siriraj Hospital, Bangkok, Thailand were enrolled in this study. Twenty-four non-NPC controls were twenty-three patients with laryngotracheal stenosis and one patient with chronic tonsillitis. Nasopharyngeal tissue biopsies of approximately 30 mg

and blood samples were collected from patients and controls prior to any clinical treatment following the protocol and with written informed consents. Tissue samples and sera were kept at -70°C and -20°C, respectively until tested.

Preparation of clinical samples

The frozen tissue biopsies were thawed and DNA was extracted by the modified procedure described by Feinmesser *et al.*[26] Briefly, 0.5-1 ml of lysis buffer C (1xPCR buffer (20 mM Tris pH 8.3, 50 mM KCl, 2 mM $MgCl_2$), 0.45% Nonidet-P40, 0.45 % Tween 20, and 100 µg of proteinase K (Sigma-Aldrich, Singapore) per milliliter) was used to digest the biopsies for 4 hours at 56°C and boiled at 95°C for 10 minutes. Two percent of the total volume of the cell lysates was directly used for DNA amplification in the nested PCR experiment.

The B95-8 (ATCC CRL 1612) and Jiyoye (ATCC CCL87) cell lines were used as the positive DNA amplification controls for EBV types A and B, respectively. These cell lines were similarly treated as tissue biopsies.

Nested PCR for detection of EBV DNA

Three sets of primers were synthesized based on the published oligonucleotide sequence primers of EBNA 2 for EBV typing.[27] The first set contained the common EBNA 2 primers (5′-AGGGATGCCTGGACACAAGA-3′ and 5′-TGGTGCTGCTGGTGGTGGCAAT-3′) which yielded 596 base pairs (bp) amplified product for both types of EBV. The second set contained the EBV type A specific primers (5′-TCTTGATAGGGATCCGCTAGGATA-3′ and 5′-ACCGTGGTTCTG GACTATCTGGATC-3′) which yielded 497 bp amplified product; and the third set contained EBV type B specific primers (5′-CATGGTAGCCTTAGGACATA-3′ and 5′-AGACTTAGTTGATGC CCTAG-3′) which yielded 150 bp amplified product.

Target DNA was first amplified in 50 µl of a reaction mixture containing 10-20 µl of the sample; 25 pmole of each primer from the first set; 400 µM of each deoxynucleoside triphosphate (dNTP); 1xPCR buffer and 1 unit of *Taq* DNA polymerase (Promega, Madison, WI, USA) for 30 cycles (denaturation for 1.5 minutes at 94°C, primer annealing for 1 minute at 60°C, and extension for 2 minutes at 72°C) in a DNA thermal cycler (Perkin-Elmer, Norwalk, CT, USA). One microliter of the PCR product was then used for the nested reaction for EBV typing

under the same condition as mentioned above except using the second and the third sets of primers. After the nested reaction, 20 μl of the DNA amplified product was analyzed by electrophoresis on 2% agarose gel, stained with ethidium bromide solution and visualized under UV light.[27] Amplification of β-globulin gene using primers described elsewhere[28] was used to confirm the presence of cellular DNA in the treated tissue.

Immunofluorescence test

The B95-8 cell line was used as a source of VCA. Details of B95-8 culture, slide preparation and staining procedure were described elsewhere.[16] The test sera were serially two fold diluted in PBS starting at 1:10. FITC-conjugated goat anti-human IgG or IgA heavy chain specific antibodies (Hyland Diagnostics, IL, USA) were used as the secondary antibody. The titer was defined as the reciprocal of the highest dilution that gave a positive reaction. A titer of $\geq$ 10 is considered to be positive.

Statistical analysis

Data were analyzed with statistical software (SPSS, Chicago). Two independent groups were compared by the Mann-Whitney U test. The level of critical significance was assigned at $p < 0.05$. Sample proportions were compared by X^2 test. When the minimum estimated expected value was < 5, Fisher's exact test was used. The validity of a screening test was evaluated in terms of sensitivity and specificity using clinical diagnosis with histologically confirmed NPC as a gold standard.

RESULTS

The tissue biopsies from the NPC patients were analysed into distinct histological types according to the WHO classification. Three (5.2%), 39 (67.2%) and 16 (27.6%) were classified as WHO type 1 (squamous cell carcinoma), WHO type 2 (non-keratinizing carcinoma) and WHO type 3 (undifferentiated carcinoma), respectively.

EBV DNA was detected in 56 (96.5%) of 58 NPC patients: 48 (82.7%) with type A, 6 (10.3%) with type B, and 2 (3.5%) with both types (Table 1). There was an association between EBV DNA detection and NPC (X^2 test at $p < 0.001$). However, EBV DNA was also found in 15

(62.5%) of 24 non-NPC controls in which 14 (58.3%) were type A. The sensitivity and specificity of EBV DNA detection by PCR for NPC patients were 96.6% and 37.5%, respectively.

All NPC patients and controls showed specific VCA IgG antibody in their sera (Table 2). Specific VCA IgG titers of NPC patient sera were significantly higher than those of control sera (Mann-Whitney U test at $p = 0.006$). The GM titers were somewhat higher in the NPC patients. However, 45 (77.6%) of 58 NPC patient sera and 10 (41.7%) of 24 control sera had IgG titers of 640 or above. There was an association between VCA IgG titers of $\geq$ 640 and NPC (X^2 test at $p = 0.004$). The sensitivity and specificity of anti-VCA IgG testing with titers of $\geq$ 640 for NPC patients were 77.6% and 58.3%, respectively. Specific VCA IgA antibody was detected in the sera of 52 (89.7%) of 58 NPC patients but only 4 (16.7%) of 24 control sera (Table 3). The specific IgA titers of NPC patient sera were significantly higher than those of control sera (Mann-Whitney U test at $p < 0.001$). The GM titers were somewhat higher in the NPC patients. There was a highly association between VCA IgA detection and NPC (X^2 test at $p < 0.001$). The sensitivity and specificity of VCA IgA detection for NPC patients were 89.7% and 83.3%, respectively.

DISCUSSION

NPC is strongly related to EBV infection.[5] All histological types of NPC were present in this study; however, only 5.2% were WHO type 1 since this type was not common in Thailand.[7,29] Laboratory diagnosis of EBV infection for NPC has been based on serological assays[13,30-32] and recently on EBV DNA detection by PCR.[20-22] In this study, EBV DNA was detected in 96.5% (56/58) of NPC patients and 62.5% (15/24) of controls, both with predominantly type A. The percentage of EBV DNA detection in NPC patients in this study was similar to that of our previous report[7] in which EBV DNA was detected in 94.1% (32/34) of the cases. However, EBV DNA was found in only 20% (1/5) of the non-NPC controls. The higher number of EBV DNA detection in the non-NPC controls in the present study (62.5% vs 20%) may due to the larger number of controls and a more sensitive PCR technique used. This high number of positive EBV DNA in the controls was similar to the previous findings of other groups, i.e., 70%-80% of the nasopharyngeal tissues from healthy individuals were shown to have EBV

DNA by PCR technique.[23,24] In addition, Diaz-Matoma, *et al.*[25] reported that 56% of healthy individuals had detectable levels of EBV DNA in oropharyngeal cells. These findings, including ours, support the concept that although EBV has been implicated in the pathogenesis of NPC, other factors such as environmental and genetic factors must be considered to be involved in the etiology of NPC. EBV infection is ubiquitous in all human population. The virus replicates in epithelial cells and establishes a latent infection in lymphoid cells. Oropharyngeal epithelial cells are the source of the virus found in the throat.[33,34] Nasopharyngeal tissues in non-NPC individuals could possibly be infected with EBV that is periodically excreted in the oropharynx. The specificity of the EBV DNA detection in this present study was only 37.5%. Therefore, we conclude in this study that EBV DNA detection by PCR might not be a suitable marker for screening NPC.

On the other hand, we demonstrated that the titers of both serum IgG and IgA antibodies against EBV VCA in the NPC patients were significantly higher than those of the non-NPC controls. These results were similar to those of our previous study.[16] Although serum IgG anti-VCA was found in non-NPC controls with various titers, the GM titers were somewhat lower in the controls. The GM titer in this study was similar to that of our previous report (457.1 and 371.5, respectively). In this study, we found that the serum IgG anti-VCA titer of $\geq$ 640 was associated with NPC. However, the specific VCA IgG antibody titer of $\geq$ 640 may not be used as a marker for screening NPC because of the low specificity (58.3%). Interestingly, the findings that serum IgA anti-VCA antibody found in 89.7% (52/58) of NPC patients but only in 16.7% (4/24) of the controls were similar to 84.6% (77/91) in the NPC patient sera and 15.5% (22/142) in the control sera reported in our previous study.[13] This specific IgA anti-VCA antibody was highly associated with NPC. This result is consistent with earlier studies showing a relationship between the specific IgA antibody and NPC.[35,36]

In conclusion, EBV DNA detection by PCR may not be a useful tool for screening NPC because of its low specificity. On the other hand, the high specificity and sensitivity of specific IgA anti-VCA antibody appears to confirm the use of this antibody as a marker for screening or diagnosis of NPC.

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Table 1. Different PCR specific EBV in biopsy tissues from NPC patients and controls

Histological types	No. biopsy tissues (%)	No. biopsy tissues with PCR specific EBV			
		type A (%)	type B (%)	type A+B (%)	not found (%)
NPC					
WHO type 1	3 (5.2)	2	1	0	0
WHO type 2	39 (67.2)	33	3	1	2
WHO type 3	16 (27.6)	13	2	1	0
Total	58	48 (82.7)	6 (10.3)	2 (3.5)	2 (3.5)
Controls	24	14 (58.3)	1 (4.2)	0	9 (37.5)

Table 2. EBV specific VCA IgG antibody in NPC patients and controls

Group	EBV type specific	Total	No. cases at reciprocal titer of												GM titer
			<10	10	20	40	80	160	320	640	1280	2560	5120	10240	
NPC	A	48	0	0	0	0	0	3	7	16	14	4	3	1	871.0
	B	6	0	1	0	0	0	2	0	3	0	0	0	0	199.5
	A+B	2	0	0	0	0	0	0	0	0	1	0	1	0	2570.4
	Not found	2	0	0	0	0	0	0	0	1	0	1	0	0	1288.2
	Total	58	0	1	0	0	0	5	7	20	15	5	4	1	955.0
Controls	A	14	0	0	0	0	0	2	6	3	0	3	0	0	524.8
	B	1	0	0	0	0	0	0	0	1	0	0	0	0	640.0
	A+B	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	Not found	9	0	0	0	0	0	2	4	2	1	0	0	0	371.5
	Total	24	0	0	0	0	0	4	10	6	1	3	0	0	457.1

Table 3. EBV specific VCA IgA antibody in NPC patients and controls

Group	EBV type specific	Total	No. cases at reciprocal titer of												GM titer
			<10	10	20	40	80	160	320	640	1280	2560	5120	10240	
NPC	A	48	5	9	6	7	13	4	4	0	0	0	0	0	36.3
	B	6	0	0	1	2	2	1	0	0	0	0	0	0	56.2
	A+B	2	0	0	0	1	0	1	0	0	0	0	0	0	79.4
	Not found	2	1	0	0	1	0	0	0	0	0	0	0	0	14.1
	Total	58	6	9	7	11	15	6	4	0	0	0	0	0	38.0
Controls	A	14	12	1	0	0	1	0	0	0	0	0	0	0	6.5
	B	1	1	0	0	0	0	0	0	0	0	0	0	0	5.0
	A+B	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	Not found	9	7	1	1	0	0	0	0	0	0	0	0	0	6.3
	Total	24	20	2	1	0	1	0	0	0	0	0	0	0	6.3

Manuscript 2

Specific IgA Antibody to Epstein-Barr Viral Capsid Antigen : a Better Marker for Screening Nasopharyngeal Carcinoma

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a running title: specific EBV-VCA IgA antibody for screening NPC

Key word: nasopharyngeal carcinoma (NPC); Epstein-Barr virus (EBV); specific viral capsid antigen (VCA) IgA antibody; polymerase chain reaction (PCR)

Summary

Nasopharyngeal carcinoma (NPC) is strongly associated with Epstein-Barr virus (EBV). To assess whether EBV DNA detection by polymerase chain reaction (PCR) or specific antibody to EBV viral capsid antigen (VCA) was a better marker for screening NPC, nasopharyngeal tissues and blood samples from 58 NPC patients and 24 non-NPC controls were tested for the presence of EBV DNA and serum specific VCA antibodies, respectively. EBV DNA was detected in 56 (96.5%) of NPC patients and 15 (62.5%) of controls, with predominant EBV type A in both groups. On the other hand, specific VCA IgA antibody was detected in the majority of NPC patients: 52 (89.7%) but only 4 (16.7%) were detected in controls. Therefore, specific VCA IgA antibody may serve as a better marker for screening NPC than EBV DNA detected by PCR.

Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common cancers in Southern China and South-East Asia including Thailand.[1-2] Epstein-Barr virus (EBV) has been found to be closely associated with this cancer.[3] There are two different types of EBV: types A and B (or types 1 and 2). Although both types have been reported in nasopharyngeal tissues, type A is more common.[4] Different sequences in the coding region of Epstein-Barr nuclear antigen-2, 3, 4 and 6 (EBNA-2, 3, 4 and 6) were used to distinguish type A from type B.[5]

Antibodies to EBV antigens have been used for the diagnosis of NPC. Moreover, IgG- and IgA-anti VCA antibody titer in NPC patients were demonstrated to be higher than that of healthy cases.[7] Meanwhile, detection of EBV DNA in tissue by polymerase chain reaction (PCR) has recently been used for screening for NPC.[8] The purpose of this study was to evaluate whether specific anti VCA antibody or EBV DNA detection by PCR was a better marker for screening patients with NPC.

Materials and Methods

Nasopharyngeal tissue biopsies and blood samples were collected prior to any clinical treatment in compliance with an informed consent protocol from 58 NPC patients and 24 non NPC controls attending at Department of Otolaryngology, Siriraj Hospital, Bangkok, Thailand. Preparation of tissue biopsies,[9] EBV DNA type specific,[5] and IgA-, IgG-anti-VCA antibody titers[10] were performed as described elsewhere.

Results

The tissue biopsies from the NPC patients were analysed into distinct histological types according to the WHO classification. Three (5.2%), 39 (67.2%) and 16 (27.6%) were classified as WHO type 1 (squamous cell carcinoma), WHO type 2 (non-keratinizing carcinoma) and WHO type 3 (undifferentiated carcinoma), respectively.

EBV DNA was detected in 56 (96.5%) of 58 NPC patients: 48 (82.7%) with type A, 6 (10.3%) with type B, and 2 (3.5%) with both types. However, EBV DNA was also found in 15 (62.5%) of 24 non-NPC controls in which 14 (58.3%) were type A. All NPC patients and controls showed specific VCA IgG antibody in their sera. Specific VCA IgG titers of NPC patient sera were significantly higher than those of control sera (Mann-Whitney U test at $p = 0.006$). The GM titers were somewhat higher in the patients. However, 45 (77.6%) of 58 NPC patient sera and 10 (41.7%) of 24 control sera had IgG titers of 640 or above. Specific VCA IgA antibody was detected in the sera of 52 (89.7%) of 58 NPC patients but only 4 (16.7%) of 24 control sera. The specific IgA titers of NPC patient sera were significantly higher than those of control sera (Mann-Whitney U test at $p < 0.001$). The GM titers were somewhat higher in the patients.

Conclusion

All histological types of NPC were present in this study; however, only 5.2 % were WHO type 1 since this type was not common in Thailand. In this study, EBV DNA was detected in 96.5% (56/58) of NPC patients and 62.5% (15/24) of controls, both with predominantly type A. This high number of controls in which EBV DNA was detected supports the concept that although EBV has been implicated in the pathogenesis of NPC, other factors such as other

environmental and genetic factors must be considered to be involved in the etiology of NPC. EBV infection is ubiquitous in all human population. The virus replicates in epithelial cells and establishes a latent infection in lymphoid cells. Oropharyngeal epithelial cells are the source of the virus found in the throat. Nasopharyngeal tissues in non-NPC individuals could possibly be infected with EBV that is periodically excreted in the oropharynx. Therefore, we conclude in this study that EBV DNA detection by PCR might not be a suitable marker for screening NPC.

On the other hand, we demonstrated that the titers of both IgG and IgA antibodies against EBV VCA in NPC patient sera were significantly higher than those of control sera. However, IgG anti-VCA titer of $\geq$ 640 was also demonstrated in 41.7% of control sera. The specific VCA IgG antibody titer of $\geq$ 640 may not be used as a marker for screening NPC. Interestingly, IgA anti-VCA antibodies were detected in 89.7% (52/58) in NPC patient sera but only 16.7% (4/24) in control sera. This specific IgA anti-VCA antibody was highly associated with NPC. Our results appear to confirm the use of specific IgA anti-VCA antibody for screening or diagnosis of NPC.

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