Prevalence of Type Specific Epstein-Barr Virus in the Genital Tract of Genital Herpes Suspected Patients

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Abstract

A total of 62 clinical specimens from the genital tract of patients who were suspected of contracting genital herpes were investigated for HSV infection by the virus isolation method, and also investigated for the co-infection with EBV infection by detecting EBV DNA using nested PCR. HSV infection was diagnosed in 30 (48.4%) of the study cases, and so was EBV. EBV DNA was present in 17 (56.7%) of the 30 HSV positive samples. No correlation was found between the co-existence of these two viruses together. EBV DNA was detected in genital specimens of cervical, vaginal, urethral, and anal swabs. Ninety per cent of EBV belonged to type 1, and the remainder belonged to type 2 and mixed types. The role of EBV in genital tract infection needs to be further investigated.

Key word : Epstein-Barr Virus, Genital Tract, Genital Herpes

Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus which causes an asymtopmatic infection or mild upper respiratory infection in young children, infectious mononucleosis (IM) or kissing disease in adolescents and young adults, oral hairy leukoplakia in the human imunodeficiency virus infected cases, and neoplasia such as nasopharyngeal carcinoma and lymphomas in certain populations[1]. The virus spreads mainly by saliva, but also by blood and organ transplants. The evidence for the presence of EBV on the genital mucosa and in genital secretions also raised the possibility for the mode of a sexual transmission[2-6]. EBV has been shown to produce genital ulcers in patients with IM. However, the infection may be subclinical. Prevalence of EBV in the genital tract of patients of both genders who attended the Sexually Transmitted Disease Clinic (STD) or the Health Service as reported by various investigators varied from 11-48 per cent[4-6]. The aims of this study was to use the technique of polymerase chain reaction (PCR) for determining the prevalence of EBV infection in the genital tract of male and female patients who attended the STD Clinic, Siri-

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raj Hospital with symptoms suspicious of genital herpes. Moreover, EBV subtypes are also identified by this PCR technique.

MATERIAL AND METHOD

Specimen collection and processing

A total of 62 clinical samples consisting of 23 cervical swabs, 9 vaginal swabs, 11 anal swabs and 19 urethral swabs were obtained from patients of both genders who visited the STD Clinic, Department of Obstetrics and Gynecology and Department of Dermatology, Faculty of Medicine Siriraj Hospital. All specimens were collected in viral transported media which were composed of Hank's Balance Salt solution (Grand Island Biological Company, New York, U.S.A.) supplemented with 0.4 per cent fetal bovine serum (FBS) (GIBCO), 200 units/ml penicillin, 20 µg/ml gentamycin and 1 µg/ml fungizone and sent to the Division of Virology, Department of Microbiology for laboratory investigation.

The specimens were centrifuged at 1,500 rpm for 15 minutes at 4°C, then the supernatants were harvested and divided into two aliquots. The first aliquot was used for isolation of HSV in Vero cell culture ; and the second aliquot was stored frozen at -20°C and used for detection of EBV DNA by nested PCR.

Isolation of HSV in Vero cell culture

Vero cell cultures were grown in the growth medium containing Minimum Essential Medium (MEM) (GIBCO) plus 10 per cent FBS, antibiotics and fungizone. The growth medium was replaced with the maintenance medium (MEM supplemented with 2 per cent FBS, antibiotics and fungizone) prior to specimen inoculation. Duplicate tubes of Vero cell monolayer were inoculated with each inoculum of 200 µl of clinical sample, then, incubated at 37°C and observed daily for 5-7 days for appearance of cytopathic effect (CPE) characteristics of HSV infection, i.e., clumps of round and refractile infected cells with occasional presence of multinucleated giant cells. The cultures with CPE were confirmed for HSV infection by indirect immunofluorescence staining with specific anti-HSV antibody (Dakopatts, Glostrup, Denmark).

Nested PCR for detection of EBV DNA

Oligonucleotide primers

Three sets of primers were synthesized based on the published oligonucleotide sequence primers of EBNA2 for EBV typing[7]. The first set contained the common EBNA2 primers (5' -AGGG ATGCCTGGACACAAGA-3' and 5' -TGGTGCTGC TGGTGGTGGCAAT-3' which conferred 596 base pairs (bp) PCR product for both types of EBV. The second set contained the EBV type 1 specific primers (5' -TCTTGATAGGGATCCGCTAGGATA-3' and 5' -ACCGTGGTTCTGGACTATCTGGATC-3') which conferred 497 bp product ; and the third set contained EBV type 2 specific primers (5' -CATGG TAGCCTTAGGACATA-3' and 5' -AGACTTAGT TGATGCCCTAG-3') which conferred 150 bp product.

PCR cycles

Target DNA was first amplified in 50 µl of a reaction mixture containing 10 µl of sample; 25 pmole of each primer from the first set; 400 µM of each deoxynucleoside triphosphate (dNTP); buffer (20 mM Tris pH8.3, 50 mM KCl, 2 mM $MgCl_2$) and 1 unit of Taq polymerase (Promega, Madison, WI, U.S.A.) for 30 cycles, including 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, U.S.A.). One microliter of each PCR product was then used for the nested reaction for EBV typing under the same condition mentioned above. After the nested reaction, 20 µl of the DNA amplified product was analyzed by electrophoresis on 2 per cent gel agarose, stained with ethidium bromide solution and visualized under UV light.

Treatment of clinical samples

The frozen samples were thawed and boiled at 95°C for 10 minutes before being used in the nested PCR experiment.

EBV DNA positive controls

Lysates of B95-8 (EBV type 1 transformed cell line; ATCC CRL 1612) and Jiyoye (EBV type 2 transformed cell line; ATCC CCL87) were used as the positive amplification controls, and HSV-infected Vero cells were used as the negative amplification controls. Viral transformed or infected cell culture was treated by modifying the procedure described by Feinmesser et al[8]. Briefly, 0.5-1 ml of lysis buffer C (consisting of 1xPCR buffer, 0.45% Nonidet-P40, 0.45 % Tween 20, and 100 µg of proteinase K per milliliter) was used to digest the cells for 4 hours at 56°C and boiled for 10

minutes, and then the cell lysates were amplified by the PCR.

RESULTS

EBV DNA was detected in 30 (48.4%) of 62 specimens from the genital tract : 12 (52.2%) of 23 cervical swabs, 4 (44.4%) of 9 vaginal swabs, 9 (47.4%) of 19 urethral swabs and 5 (45.5%) of 11 anal swabs as shown in Table 1. HSV was isolated from 30 (48.4%) of 62 specimens studied in which 17 (56.7%) of these HSV positive samples were also positive for EBV DNA. In addition, EBV DNA was also detected in 13 (40.7%) of the 32 HSV negative samples. Statistical analysis showed no significant correlation between presence of EBV infection and presence of HSV infection (Chi-square test = 0.58; $p > 0.05$).

Prevalence of EBV type specific infection was determined from the 30 EBV positive samples; and it was shown that 27 (90%) samples were of EBV-1, 1 (3.3%) were of EBV-2 and 2 (6.7%) were a mixture of both types. The sample size was not large enough to determine site prevalence of certain types of specific infection (Table 2).

DISCUSSION

EBV DNA have been reported to be detected in the clinical specimens collected from the cervix, urethra and anal mucosa at the frequency of 28-42 per cent, 48 per cent and 9-30 per cent, respectively[4-6]. Those results were similar to what has been observed in our study which showed the presence of EBV DNA in 48 per cent of the specimens collected from the genital tract : 52, 44, 47 and 46 per cent for cervical, vaginal, urethral and anal swabs, respectively. We also showed that 90 per cent of EBV present in the genital tract was of type 1, while the remainder were of EBV-2 and the mixed infection between two EBV types. The demonstration of EBV DNA in the cell free fraction of the clinical samples in our study reflected the presence of EBV virions in the genital tract and thus, confirmed the earlier work done by other investigators on the ability of this virus to be transmitted by sexual route[5,6].

Several reports have indicated that protein products of one herpesvirus could activate the other latent herpes members[8-10]. Based on this information, we speculate that EBV may reactivate

Table 1. Prevalence of detectable EBV DNA and HSV isolation from genital specimens.

Sample	No. EBV positive/ No. tested (%)		HSV isolation in EBV positive cases			
			HSV Positive		HSV Negative	
Cervical swab	12/23	(52.2)	6/7	(85.8)	6/16	(37.5)
Vaginal swab	4/9	(44.4)	1/2	(50)	3/7	(42.9)
Urethral swab	9/19	(47.4)	6/13	(46.2)	3/6	(50)
Anal swab	5/11	(45.5)	4/8	(50)	1/3	(33.3)
Total	30/62	(48.4)	17/30	(56.7)	13/32	(40.7)

Table 2. EBV subtypes present in genital specimens.

Sample	No. of samples with EBV DNA	EBV-1 (%)		EBV-2 (%)		Mixed types (%)	
Cervical swab	12	11	(91.7)	1	(8.3)	0	
Vaginal swab	4	4	(100)	0		0	
Urethral swab	9	7	(77.8)	0		2	(22.2)
Anal swab	5	5	(100)	0		0	
Total	30	27	(90)	1	(3.3)	2	(6.7)

the latent HSV or *vice versa*. Our study also demonstrated the co-presence of EBV and HSV in the same clinical specimen such that 57 per cent of samples positive for HSV were also EBV positive. However, statistical analysis did not show a significant correlation on the co-existence of these two viruses together.

EBV in the genital tract may be released from the reactivated, infected B-lymphocytes migrating into the inflammatory sites occurring from any infection. In addition, EBV has been shown to infect the epithelial cells lining the genital mucosa and cause productive infection. EBV was shown to produce genital ulcers in cases with IM. Therefore, the role of EBV as a causative agent of STD should come into account if the common pathogens have been excluded. And because of its ability to transform cells, the role of EBV as a co-factor of human papilloma virus in the development of uterine cervical carcinoma remains to be investigated.

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อัตราความชุกของเชื้อไวรัสเอ็บสไตน-บาร์ทัยป์ต่างๆในบริเวณอวัยวะสืบพันธุ์ของผู้ป่วยที่สงสัยติดเชื้อเริม

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คณะผู้วิจัยได้ทำการสำรวจอัตราความชุกของการติดเชื้อไวรัสเอ็บสไตน์บาร์ (อีบีวี) ในผู้ป่วยที่สงสัยการติดเชื่อเริมบริเวณอวัยวะสืบพันธุ์ การตรวจทางห้องปฏิบัติการสำหรับบ่งชี้การติดเชื้อเริมและเชื้ออีบีวีนั้น ใช้วิธีการแยกเชื้อและการหาดีเอ็นเอด้วยวิธีพีซีอาร์ ตามลำดับ สิ่งส่งตรวจจากผู้ป่วยจำนวน 62 ราย พบการติดเชื้อเริมและการติดเชื้ออีบีวี เท่ากัน คือ 30 ราย (48.4%) ในผู้ติดเชื้อเริม 30 รายนี้ตรวจพบดีเอ็นเอของเชื้ออีบีวี ในผู้ป่วยจำนวน 17 ราย (56.7%) และไม่พบความสัมพันธ์ระหว่างการตรวจพบเชื้อเริมและเชื้ออีบีวีร่วมกัน ดีเอ็นเอของเชื้ออีบีวีสามารถตรวจพบในสิ่งส่งตรวจที่เก็บจากบริเวณอวัยวะสืบพันธุ์ เช่น cervical, vaginal, urethral และ anal swabs โดยพบว่าประมาณเก้าสิบเปอร์เซนต์เป็นดีเอ็นเอของเชื้ออีบีวี ทัยป์ 1 ส่วนที่เหลือเป็นอีบีวี ทัยป์ 2 และ ทัยป์ 1 ร่วมกับทัยป์ 2 สำหรับบทบาทของเชื้ออีบีวีต่อการติดเชื้อในอวัยวะสืบพันธุ์ยังต้องศึกษาต่อไป

คำสำคัญ : ไวรัสเอ็บสไตน-บาร์, อวัยวะสืบพันธุ์, เริมบริเวณอวัยวะสืบพันธุ์

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