Prevalence of Herpes Simplex Virus Infection in Patients Suspected of Genital Herpes ; and Virus Typing by Type Specific Fluorescent Monoclonal Antibodies

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Abstract

During the period between April 1994 and February 1996, a total of 154 female patients who attented the Clinic of Female Sexually Transmitted Diseases, Siriraj Hospital with clinical symptoms suspected of genital herpes were investigated for herpes simplex virus (HSV) infection by the virus isolation method in Vero cell cultures. Swabs from external genital lesions and the cervix from each patient were collected separately and used as the clinical specimens for isolation of HSV. The virus isolates were identified by indirect immunofluorescence (IIF) staining of the infected cell cultures using polyclonal HSV-2 specific antiserum which was reactive to common HSV antigens for both types of viruses. Typing of HSV was performed by direct IF using monoclonal antibody specific to HSV-1 or HSV-2. HSV was isolated from 78.6 per cent (121 of 154) of the cases studied; and among the infected cases, there were 47.9 per cent (58 of 121) in whom the infection involved both external genital lesions and cervixes, and 50.4 per cent (61) in whom the infection was limited to external genital lesions only. There were 2 cases (1.7%) in whom HSV was isolated from cervixes but not external genital lesions. Seventy-five HSV isolates were further subjected to typing. The present study showed that HSV-1 was accounted for 18.7 per cent (14 isolates), while HSV-2 took the remaining part of 81.3 per cent (61 isolates). The data demonstrated an increase in the prevalence of HSV-1 in genital herpes in our people.

At present, genital herpes is the most common sexually transmitted diseases in Thailand and worldwide. Lesions typical of genital herpes are the presence of a crop of vesicles at the genitalia which progress to ulceration at late disease stage. Most often local lesions are accompanied by inguinal lymphadenopathy, and may involve urethritis or cervicitis especially in cases of primary genital herpes. A considerable overlap in clinical manifestation of genital herpes and other

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genital ulcers e.g., chancroid, syphilis, lymphogranuloma venereum and donovanosis has been recognized[1,2]. Since most of the patients attend the hospital after vesicles are broken into ulcerative lesions, laboratory diagnosis is then neccesary for the precise disease diagnosis. Among several laboratory tecniques available, isolation of the virus in cell culture is accepted as the standard diagnostic test[3].

Genital herpes is mostly caused by herpes simplex virus type 2 (HSV-2), and minor proportions are attributable to herpes simplex virus type 1 (HSV-1). Data from various investigators showed that HSV-1 accounted for 4-37 per cent of cases of first episodes of genital herpes[4-7]. Investigation on HSV type specific infection in patients with genital herpes not only provides the epidemiological data, but also provides the clinical information. Although the clinical course of first episode genital herpes caused by either HSV-1 or HSV-2 is similar, genital herpes associated with HSV-2 recurs more often than that associated with HSV-1[6,8]. And this is the reason why frequency of HSV-1 infection in primary first episode is higher than that found in the reccurrent one. Reeves, et al reported that 15 per cent of primary first episodes (no HSV antibody in acute phase sera) were caused by HSV-1 compared with 3 per cent of non-primary first episodes (present of HSV antibody in acute phase sera) and 2 per cent of recurrent episodes; and among the patients with HSV-2 genital herpes, the recurrent rate was directly related to level of neutralizing antibody in convalescent sera[6]. Moreover, drug resistant HSV-2 arises more frequently than drug resistant HSV-1[9].

The study herein determined the prevalence of HSV infection in patients suspected of genital herpes by isolation of the viruses in cell culture; and the virus isolates were then typed by direct immunofluorescence test using a panel of HSV type specific monoclonal antibodies.

MATERIAL AND METHOD

Subjects

The present study was conducted on a total of 154 child bearing age patients who attended the Clinic of Female Sexually Transmitted Diseases, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital during the period April 1994 to February 1996. These patients were suspected of genital herpes as clinical symptoms of burning sensation, crops of vesicles or shallow ulcers at the genitalia were informed. However, history of primary or recurrent episode of HSV infection was not obtained.

Specimen Collection and Processing

A swab from an external genital lesion and a swab from the cervix were collected from each patient, and placed into separate tubes containing 2-3 ml of viral transport media and carried in an ice chest to the Virology Laboratory, Department of Microbiology, Faculty of Medicine Siriraj Hospital. The tubes of clinical samples were centrifuged at 268xg for 10 minutes at 4°C, and then the supernatants were harvested and kept for isolation of virus which was performed immediately, or else the specimens would be kept at 4°C and tested within three days.

Virus Isolation Method

Vero cells were cultured in 15x50 mm glass-tubes using growth media containing minimum essential medium (MEM) (GIBCO-New York), 10 per cent fetal calf serum (FCS) (GIBCO), 100 units/ml penicillin, 100 µg /ml streptomycin and 1 µg /ml fungizone. The cell monolayers aged 1-3 days were used for isolation of HSV. Just before inoculation with 200 µl of a clinical sample, the growth medium was replaced with 0.8 ml of maintenance medium (MEM plus 2% FCS) and incubated at 37°C. The inoculated cultures in duplicate tubes were observed daily for seven days for cytopathic effect (CPE) characteristic of HSV infection i.e., appearance of foci of round-up and refractile cells with occasionally presence of multinucleated giant cells. The infected cultures usually showed CPE within 1-5 days after infection, and then they were further identified for HSV infection.

Virus Identification

The infected cultures were identified for HSV infection by indirect immunofluorescence (IIF) using rabbit polyclonal specific antiserum (Dakopatts, Glostrup, Denmark) which was reactive to both HSV types

Briefly, cells from the infected cultures showing CPE were scraped off the glass surface, pelleted and deposited on microscopic glass slides. Then, the slides were air-dried, fixed in pre-cooled

acetone at -20°C for 10 minutes before staining, or else the fixed slides could be kept frozen at -20°C until stained.

Cell deposits on the fixed slides were covered with rabbit anti-HSV antisera for 30 minutes at 37°C ; then, the slides were rinsed and soaked in phosphate buffer saline (PBS) for 10 minutes, followed by a final rinse in distilled water. Goat-anti rabbit Ig conjugated with fluorescein isothiocyanate (FITC) (Dakopatts) was applied, and the slides were incubated for another 30 minutes at 37°C followed by a rinse -soak-rinse cycle as mentioned in the first staining step. The slides were counter stained with Evan's blue before being examined under a fluorescence microscope. HSV antigens were observed in both nucleus and cytoplasm, and there was no difference in appearance of specific antigens of both HSV types.

HSV Typing

FITC conjugated-HSV type specific monoclonal antibodies (Syva MicroTrak, Palo Alto, U.S.A.) were used to type the HSV infected cultures by direct IF which employed a single staining step and a single washing cycle. All of the stained cultures could be classified either as HSV-1 or HSV-2 type specific infection.

RESULTS

Isolation of HSV from Patients Suspected of Genital Herpes

Among a total of 154 lesion swab samples and 154 cervical swab samples used for isolation of HSV in Vero cell monolayer cultures, 119 (77.3%) lesion swab samples and 60 (39.0%) cervical swab samples were positive for HSV isolation. All except two cases with positive virus isolation in cervical swab samples were also positive for virus isolation in lesion swab specimens. Among 121 HSV infected cases, there were 58 cases (47.9%) in whom HSV infection involved both external genital lesions and cervixes, and 61 cases who were positive for HSV isolation only in lesion swab specimens. Overall, there were 121 (78.6%) cases among 154 patients suspected of genital herpes who were positive for HSV infection (Table 1).

Typing of HSV Isolates

Type assignment of the 75 HSV isolates was employed by type specific monoclonal antibodies. Cross reaction between type specific antibody and heterologous virus type was not seen. It was shown that HSV-2 played a major role as the causative agent of genital herpes, i.e., HSV-2 took part of 81.3 per cent (61 isolates), while HSV-1 shared 18.7 per cent (14 isolates) of all isolates studied.

DISCUSSION

HSV infections occur throughout the world. The two distinct types namely HSV-1 and HSV-2 elicit various clinical manifestations with different organ tropism. HSV-1 is common in oro-facial, eye and brain infections, while HSV-2 is common in genital infection. In other words, HSV-1 infection is frequently found in anatomical areas above the umbilicus, and HSV-2 infection is so in areas under the umbilicus. The reverse phenomenon was occasionally seen in a minority of cases[2,10,11]. However, prevalence of HSV-1 over HSV-2 in genital herpes has also been reported by some investigators[12].

Based on virus isolation method, we were able to diagnose HSV infection in 78.6 per cent (121) of the 154 cases suspected of genital herpes.

Table 1. Isolation of HSV from clinical specimens collected from patients suspected of genital herpes (N = 154 cases)

Type of specimen positive for HSV	Number (%) positive for HSV isolation
Lesion swab alone	61 (39.6)
Cervical swab alone	2 (1.3)
Lesion swabs and cervical swabs	58 (37.7)
Total positive lesion swabs	119 (77.3)
Total positive cervical swabs	60 (39.0)
Total positive cases	121 (78.6)

Among 121 HSV infected cases, there were 47.9 per cent (58) in whom the infection involved both external genitalia and cervixes. External genital infection alone occurred in 50.4 per cent (61 cases). There were two cases in whom HSV had been isolated from cervixes but not from lesions which could be explained that lesion swab samples might be collected late. HSV infection was not diagnosed in the remaining 21.4 per cent (33) of the subjects suspected of genital herpes which can be interpreted that the disease was caused by other etiologic agents. In addition, our unpublished data showed that the more sensitive technique of polymerase chain reaction could reveal some cases of HSV infection, while virus isolation method could not.

There were some biological differences between the two types of HSV. Clinically, after HSV-1 primary genital herpes, the disease recurred less frequently than that after HSV-2 primary genital herpes. In contrast, HSV-1 oral-labial infection recurred more frequently than HSV-2 oral-labial infection[8,10]. Those findings implied that the risk of recurrence was dependent on both the viral type and anatomical site of the infection.

HSV type specific infection in genital herpes has been conducted in Thailand previously. Yoosook, et al reported 100 per cent HSV-2 infection among 126 HSV isolates obtained from cases with recurrent genital herpes; while 5.0 per cent of HSV-1 and 95.0 per cent of HSV-2 were detected in 60 cases with first episode: and overall, 1.6 per cent of genital herpes (first episode, recurrent episode and asymptomatic infection) were caused by HSV-1 infection[13].

Our first study reported a prevalence rate of 2.6 per cent (3 of 115 HSV isolates) of HSV-1 infection in cases collected from October 1986 to March 1991[14]; and the present study showed a prevalence of HSV-1 infection of 18.7 per cent. Unfortunately, records on the history of primary or recurrent episodes of genital herpes in our subjects in both studies could not be obtained. Nevertheless, a dramatic increase in prevalence of HSV-1 associated with genital herpes is clearly shown.

It is also interesting to learn that a study in Glasgow in cases collected from 1985 to 1987 showed an increase in prevalence of HSV-1 genital herpes in patients aged $\leq$ 25 years compared with cases aged $\geq$ 25 years[15]. There was no clear explanation for this change; and episode of infection was not shown in the study mentioned.

Relying on an increase in the prevalence rate of HSV-1 genital herpes, it also indirectly reflects that oral sex practice is more common than it has ever been before. This finding has to come into consideration when an intervention method to control sexually transmitted diseases in Thailand is to come into account.

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ความชุกของภาวะติดเชื้อเริมในสตรีที่สงสัยว่าจะเป็นเริมที่อวัยวะเพศ; และการแยกทัยป์ของไวรัสโดยวิธีอิมมูนเรืองแสง

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งานวิจัยนี้เป็นการศึกษาความชุกของการติดเชื้อเริมในผู้ป่วยสตรีจำนวน 154 คน ซึ่งมารับการตรวจที่หน่วยกามโรคสตรี โรงพยาบาลศิริราช ในระหว่างเดือนเมษายน 2537 ถึง กุมภาพันธ์ 2539 ด้วยอาการที่สงสัยว่าเป็นโรคเริมที่อวัยวะเพศ ตัวอย่างตรวจที่เก็บจากผู้ป่วยทุกรายเพื่อนำมาวินิจฉัยหาสาเหตุของโรค คือ ไม้ป้ายจากปากมดลูก และไม้ป้ายจากรอยโรคที่อวัยวะเพศภายนอก ห้องปฏิบัติการไวรัสได้ทำการวินิจฉัยโรคด้วยวิธีการแยกเชื้อไวรัสในเซลล์เพาะเลี้ยง และพิสูจน์เชื้อไวรัสที่แยกได้ด้วยวิธีอิมมูนเรืองแสงโดยอ้อม (อินไดเร็ค อิมมูโนฟลูออเรสเซนส์) โดยย้อมกับโพลีโคลนัล แอนติบอดี ต่อเชื้อเริม และได้นำเชื้อไวรัสที่แยกได้จำนวนหนึ่งไปทำการจำแนกทัยป์ด้วยวิธีอิมมูนเรืองแสงโดยตรง (ไดเร็ค อิมมูโนฟลูออเรสเซนส์) โดยการย้อมด้วยโมโนโคลนอล แอนติบอดีที่มีความจำเพาะต่อไวรัสเริมทัยป์ 1 หรือ ไวรัสเริมทัยป์ 2 ผลการศึกษาพบว่าผู้ป่วยที่สงสัยว่าเป็นโรคเริมที่อวัยวะเพศ มีการติดเชื้อเริมจริงร้อยละ 78.6 (121 ใน 154 ราย) โดยผู้ป่วยที่ติดเชื้อเริมร้อยละ 47.9 (58 ใน 121 ราย) จะมีการติดเชื้อทั้งที่รอยแผลและที่ปากมดลูกร่วมด้วย ส่วนผู้ป่วยร้อยละ 50.4 (61 ใน 121 ราย) มีการติดเชื้อเกิดขึ้นที่รอยแผลเพียงแห่งเดียว ผู้ป่วยร้อยละ 1.7 (2 ราย) มีเชื้อไวรัสที่ปากมดลูกแต่ไม่มีเชื้อที่รอยโรค อาจเป็นเพราะรอยโรคเก่าแล้ว เชื้อไวรัสจำนวน 75 เชื้อ จากผู้ป่วยนี้เมื่อทำการจำแนกทัยป์แล้ว พบว่าเป็นเริมทัยป์ 1 ร้อยละ 18.7 (14 เชื้อ) และเป็นทัยป์ 2 ร้อยละ 81.3 (61 เชื้อ) ซึ่งแสดงว่าความชุกของการติดเชื้อเริมทัยป์ 1 ที่อวัยวะเพศสูงขึ้นหลายเท่าเมื่อเทียบกับอดีต

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