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นิพนธ์ต้นฉบับ

Viral isolation in different stages of recurrent herpes labialis by shell vial centrifugation cell culture.

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The study of viral isolation by shell vial centrifugation cell culture in 14 patients with recurrent herpes labialis of less than 72 hours duration in out-patient department of dermatology, chulalongkorn hospital. Viral isolation was done daily until lesion became dry crust. A total of 43 specimens were collected. Herpes simplex viruses can be isolated 100% from vesiculopustular lesion, 57% from ulcerated lesion and 27% from crusted lesion. Mean viral shedding time was 3.5 days and all of the isolated HSV were antigenic type 1.

Shell vial centrifugation cell culture is the most sensitive method for detecting infectious virions. The culture yield is highest when the specimen is taken from vesiculopustular lesion.

Key words : *Recurrent herpes labialis, Shell vial centrifugal cell culture.*

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กุณฑล พิทยาธิคุณ, วิวัฒน์ ก่อกิจ, วรรณา พรรณรักษา, ภาวพันธ์ ภัทรโกศล. การเพาะแยกเชื้อไวรัสจากแผลระยะต่าง ๆ ในโรคเริมชนิดเป็นซ้ำที่ริมฝีปาก โดยวิธี เซลล์ ไวแอล เซ็นตริคฟูเกชั่น เซลล์ คัลเชอร์. จุฬาลงกรณ์เวชสาร 2538 สิงหาคม; 39(8): 593-599

ผู้รายงานได้ทำการศึกษาในผู้ป่วย 14 ราย ซึ่งได้รับการวินิจฉัยว่าเป็นโรคเริมชนิดเป็นซ้ำที่ริมฝีปาก ที่มาตรวจที่ห้องตรวจโรคผิวหนัง รพ.จุฬาลงกรณ์ โดยที่ผู้ป่วยต้องมีอาการไม่เกิน 72 ชั่วโมง และสามารถมาโรงพยาบาลทุกวัน เพื่อทำการเพาะแยกเชื้อจากแผลในระยะต่าง ๆ คือ ระยะที่เป็นตุ่มน้ำ, ระยะที่เป็นแผล และระยะที่ตกสะเก็ด การเพาะแยกเชื้อจะทำวันละครั้งจนกว่าแผลจะตกสะเก็ด สามารถเก็บตัวอย่างได้ 43 ตัวอย่าง พบว่าสามารถตรวจแยกเชื้อจากตุ่มน้ำและตุ่มหนองได้ 100%, จากแผล 57% และจากแผลระยะตกสะเก็ด 27% โดยวิธี shell vial centrifugation cell culture ระยะเวลาที่สามารถตรวจพบเชื้อไวรัสจากแผลในโรคเริมชนิดเป็นซ้ำที่ริมฝีปาก คือ 3.5 วัน เชื้อที่ตรวจพบจากตัวอย่างทั้งหมด เป็น herpes simplex virus type 1

การเพาะเชื้อโดยวิธี shell vial centrifugation cell culture เป็นวิธีที่มีประสิทธิภาพสูง การส่งตัวอย่างตรวจเพาะเชื้อเพื่อวินิจฉัยโรคเริม ควรทำในระยะที่เป็นตุ่มน้ำและตุ่มหนอง ซึ่งเป็นช่วงที่สามารถตรวจพบเชื้อได้สูง

Herpes simplex viruses, both type 1 (HSV-1) and type 2 (HSV-2) are known to cause highly contagious and painful mucous membrane or skin lesions. HSV-1 is normally responsible for the common herpetic infection of the face, oropharynx and skin of the upper part of body while HSV-2 infects the genitalia.[1,2] Although both types can cause genital herpetic infection, HSV-2 is isolated in 80 percentages of the cases.[3]

Primary herpes simplex infection occurs mainly from directly mucocutaneous contact with infected individuals. Acute febrile illness with stomatitis will be experienced by the patients and the resolution will take place in about ten days. Recurrent episodes occurs in a proportion of the population at intervals that vary tremendously in different individuals. Up to 20% of the population suffer from recurrent herpes simplex infection which commonly manifest around the mouth or on the lip.[4] Clinical features starts as grouped erythematous painful papules, turn out rapidly to be vesicular lesions and then coalesce to from a crusted lesions. It takes four days from initial symptoms to become dry crusted and the sequence takes additional 4-5 days to complete the healing. The most suitable time for isolation of the virus is 3.5 days after initial symptom.

The aim of this study is to compare the possibility of isolation the virus from various stages of disease; namely, vesiculopustular, ulcerated and crusted lesion in recurrent herpes labialis and to determine type of herpes simplex virus together with viral shedding time.

Materials and methods

Patients and Specimens. Fourteen immunocompetent patients with recurrent herpes labialis of less than 72 hours duration from out-patient department of Dermatology, Chulalongkorn Hospital, during the year of 1992 were enrolled in the present study. On the first visit, a medical history, physical examination and Tzanck test were performed in order to establish the diagnosis of recurrent herpes labialis. All the patients were followed daily and the viral culture was done on every visits until the lesions became dry crusted. The lesions were morphologically classified into vesicle, pustule, ulcerated and crusted stage. Different techniques were used to collect the specimens at various stages of disease according to the natures of the lesions; the vesicle fluid was collected in vesicle stage, the base of ulcers were scraped with cotton swabs in ulcerated stage and the crusts were removed and scraped with cotton swabs in crusted stage, respectively. All clinical specimens were placed in cold viral transport media, immediately taken to laboratory and kept frozen at-70°C

Tzanck test. The scraped cells from lesion were smeared on glass slide and left dry at room temperature. Then, the cells were stained using Giemsa's staining method and examined under light microscopy. The presence of multinucleated giant cells with neutrophils and mononuclear cells indicated herpes viruses (HSV and VZV) infection.

Cell culture. Vero cells were grown in medium (M 199 with 10% fetal bovine serum supplemented with antibiotics (penicillin and streptomycin). Maintainance medium. (M 199 with 2% fetal Bovine serum and antibiotics) was used for maintaining the cells.

HSV isolation by shell vial centrifugation cell culture (SVC). The specimens were thawed rapidly at 37°C in water bath. To mini-

mize any contaminants, the specimens were centrifuged once at 1,000 rpm for 10 minutes at 4°C. The supernatant was collected and used as the inoculum. The amount of 0.2 ml was inoculated into vial (16 x 50 mm) containing a 12 mm diameter round coverslip seeded with Vero cells (Figure 1). The tube was then centrifuged at 2500 rpm for 1 hour at 26°C. Thereafter, the inoculum was discarded and the maintainance medium was added. After 18 hours incubation, the cover slip was washed twice with phosphate buffered saline, pH 7.4 (PBS), left it dry. The cells were fixed in cold acetone at -20°C for 10 minutes and stained for the presence of infected cells (Figure 2) by indirect immunofluorescence assay (IFA). The cells on cover slip were covered with rabbit anti HSV-1, kept in moist chamber at 37°C for 30 minutes, washed with PBS 5 minutes for 3 times and then added swine antirabbit-FITC on the cover slip for another 30 minutes, washed and counter stain with evan's blue 1 : 30,000, for 5 minutes, dipped in water for 1 minute. Finally, the mouted cells were examined for HSV-specific foci under fluorescence microscopy.

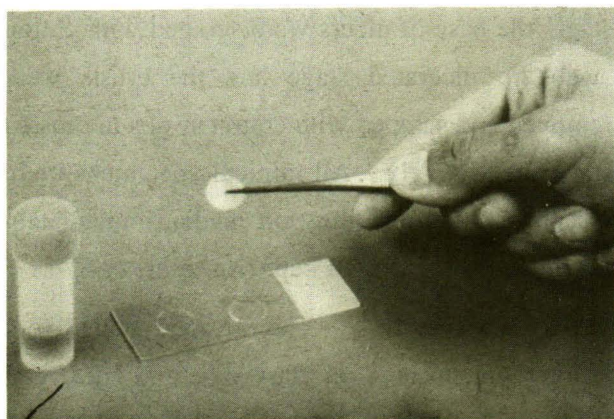

Figure 1. Shell vial, a flat base plastic tube containing a round cover slip for isolation of herpes simplex virus.

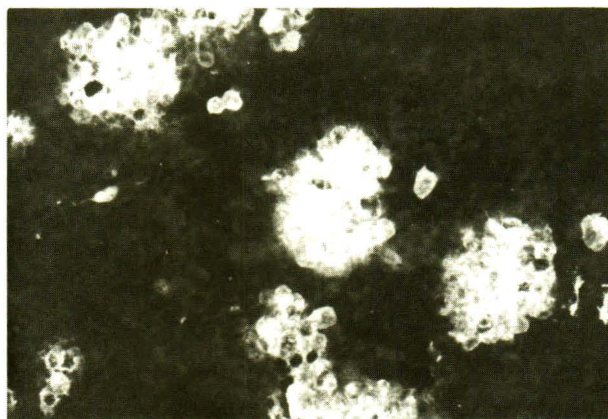

Figure 2. Positive indirect immunofluorescence shows clumping of round, swollen infected cells.

Typing of HSV by ELISA. Commercial ELISA kit (Enzygnost HSV-Ag) purchased from Behringe, Germany was used. The HSV-Ag in the specimen was trapped with anti-HSV coated well and was typed by monoclonal antibodies specific to either HSV-1 or both HSV-1 and HSV-2 conjugated with horse radish peroxidase. Thus, each sample has to perform in two wells. The determination for each type was done by comparing the optical density (OD) between these two wells.

Results

There are 13 female patients and 1 male patient enrolled in this study. They were nurses and medical personel in the hospital. All gave their consent to attend daily follow-up programme until the lesions became dry crusted.

The mean age of patients was 34.07 ± 13.51 years as shown in Table 1. The time interval between the primary infection and the recurrent episode widely ranged from one year to ten years. After initial symptom, the patients spent

2.29 ± 0.47 days by average before coming to see the doctor. The mean crusting time was 4.23 ± 0.97 days (Table 2) and mean healing time was 8.53 ± 1.39 days. The most common site of lesions was on the vermilion of the lip.

Table 1. General characteristic of patients.

		Mean value
Age		34.07 ± 13.51
Duration from first episode	(Year)	1 - 10
History of crusting time	(Day)	6.21 ± 1.76
History of healing time	(Day)	10.85 ± 3.16
Mean time from when lesion first noted to first visit	(Day)	2.29 ± 0.47

Table 2. Mean time of healing process and positive viral isolation.

	Mean value (day)
Crusting time	4.23 ± 0.97
Healing time	8.53 ± 1.39
Viral shedding time	1.85 ± 0.86

All 43 specimens at different stages were cultured for HSV and the results were shown in Table 3. The mean value of viral shedding time among 14 patients was 1.85 ± 0.86 days (Table 2). The culture yields were related with types of lesion from which specimens were taken. All of the specimen from vesiculopustular lesions were cultured positive while the specimens from ulcerated lesions and crusted lesions were cultured positive only 57% and 27%, respectively. Viral type determination using ELISA technique indicated that all of the isolated herpes simplex virus from recurrent herpers labialis in this study were antigenic type 1.

Table 3. Positive viral isolation in different stage of lesions.

Stage of lesion	Number	Percent
Vesicle and pustule	18/18	100
Ulcer	8/14	57
Crust	3/11	27

Discussion

Herpes simplex virus is double stranded DNA virus which is responsible for a wide range of clinical manifestations in human.[5] A rapid diagnosis for HSV infection may lead to improvement in patient care. There are various methods for HSV detection such as conventional cell culture (CCC), shell vial centrifugation cell culture (SVC), indirect immunofluorescence assay (IFA) and enzyme linked immunosorbent assay (ELISA). Using cell culture method is worth for detecting the presence of infectious virion. Thus, in the present study we used SVC method which is demonstrated to be the most sensitive and more rapid than CCC as reported by Pannarugsa V. and her colleagues.[6] In the report, SVC can detect only 10 plaque forming unit (PFU) of virions compared with CCC and ELISA which required at least 10^2 and 10^3 PFU, respectively.[6]

Fourteen recurrent herpes labialis patients were recruited and the specimens from their lesions were collected daily until the lesions were dry crust. From 43 specimens collected, The average viral shedding time starting from first visit of each patient was 1.85 $\pm$ 0.86 days (Table 2). However, mean time from when lesions first noted to first visit was 2.29 $\pm$ 0.47 days. Then, the actual viral shedding time (a duration that the infectious virions present in the lesions) was approximately 3.5 days. This viral shedding time is concurrent with previoused report.[7,8] Moreover, crusting time and healing time were about 4 days and 8 days, respectively (Table 2).

The efficiency of SVC method was 100% detection in vesiculopustular lesion but it was reduced to 57% and 27% in ulcerated and crusted lesions, respectively (Table 3). This was due to the declining of viable virions in those lesions. Therefore, the best specimen for detection of infectious virion should be collected from vesiculopustular lesion and clinical specimen should be placed in cold viral transport media and immediately taken to laboratory. For the best yield of viral culture. However, ulcerated and crusted lesions were also useful sources for HSV antigen detection eg. IFA technic.[9]

Typing of HSV isolated from 14 herpes labialis cases were HSV antigenic type 1, convincing that HSV-1 still be a major causative agent of recurrent herpes labialis in Thai population.

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