# Increase of Genital HSV-1 And Mixed HSV-1 and HSV-2 Infection in Bangkok, Thailand

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*From January 1998 to December 2004, 207 out of 1,125 samples were HSV isolation positive and typed. Two groups of patients, Thai and foreigner, as well as site of infection, non-genital and genital area, were identified. The prevalence of non-genital HSV-1 infection from 27 samples of Thai patients was 81.84%. Out of 180 genital samples, 39.02% HSV-1 and 43.09% HSV-2 from 123 Thai patients and 36.84% HSV-1 and 49.12% HSV-2 of 57 foreigner patients were determined. Moreover, mixed infection of HSV-1 and HSV-2 was found in both Thai and foreigner groups, 17.89% and 14.04%, respectively. The prevalence of genital HSV-1 infection in Thai patients chronologically increases from 1.6% to 56.91% from of 1985 to 2004. Increase of HSV-1 genital infection and mixed HSV-1 and HSV-2 infection in Thai people might probably be due to changing of sexual behavior in the AIDS era.*

**Keywords:** *HSV-1, HSV-2, Genital infection, Thailand*

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Herpes simplex virus (HSV) is one of significant pathogens in public health worldwide. It is a member of the Herpesviridae family, alphaherpesvirinae subfamily. HSV is divided into 2 types, type 1 (HSV-1) and type 2 (HSV-2). Both types share extensive nucleic acid sequence homology the fifty percent[1]. Although HSV infections are mostly asymptomatic infection but in some people, after the first episode of infection following repeated recurrent infections time to time, it becomes annoying and under some circumstances it may provoke to life-threatening diseases especially in immunocompromised hosts such as newborn babies, transplantation patients who received immunosuppressive drug, and HIV infected patients[2-4]. Beside host immunity status, anatomic sites of HSV infection as well as type of viruses are also play role in the severity of the symptom[5]. HSV-1 is usually transmitted from person to person by direct contact with saliva and respiratory secretion which associated with infection of the lips, mouth, face and skin lesions above the waist while HSV-2 is commonly transmitted by sexual contact and causes infection of genital area[6,7]. However, this tropism of the virus seems to have changed. The increasing of HSV-1 infection at genital area was chronologically detected. The prominence of HSV-1 genital infection has been variably reported worldwide as between 6-78%[8-14]. In Asia, since early 1970, genital herpes caused by HSV-1 was indicated to be greater frequency in Japan than in the United State. That was 50% of HSV strains recovered from the genital sites was of the HSV-1 subtype[15,16]. Unlike to Thailand, Yooksook, et al in 1989[17] reported HSV-1 isolated from female patients with genital lesion represented from 1985 to 1986 was only 1.6%. Puthavathana, et al showed the prevalence rate of HSV-1 infection in all genital cases collected from 1986 to 1991 and 1994 to 1996 was 2.6 and 18.7%, respectively [18,19]. All of theses studies were performed in hospitals located in Bangkok, capital of Thailand. Thus, aim of the present study was to survey any change in prevalence of HSV infection in Thailand especially genital herpes.

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## Material and Method

### *Specimens*

A number of 1,125 specimens was obtained from Virology Unit, Department of Microbiology, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Bangkok, Thailand during 1 January 1998 to 31 December 2004. All of them were requested for

HSV isolation.

### *HSV isolation and identification*

Isolation of HSV was done in Vero cells, grown in M199 Earle's salt supplemented with 10% fetal bovine serum, 100 unit/ml of Penicillin and 100 ug of Streptomycin (All reagents were purchased from GIBCO BRL, USA) using shell vial centrifugation method. In brief, 0.2 ml of sample was inoculated into Vero cell grown on the 12 mm diameter round coverslip in shell vial tube (16X50 mm). After one hour centrifugation at 2500 rpm at 26 ºC, the cells were washed once with phosphate buffered saline (PBS), pH 7.4 and the medium was replaced. After incubating at 37 ºC for 14-16 hours, the cells were washed, air-dried and fixed in cold acetone (-20 ºC) for 10 minutes. The acetone-fixed cells were then further stained for the presence of HSV infected cells by indirect immunofluorescent assay using rabit polyclonal antibody against HSV-1 (DAKO A/S, Denmark) following with goat anti- rabbit IgG FITC conjugated antibody (DAKO A/S) and counterstained with Evan's blue. The stained cells were examined under fluorescence microscopy (Olympus, Japan). All positive specimens were kept at -70 ºC until use.

### *HSV typing*

All of those positive samples were re-cultured in Vero cells for further typing by indirect immunofluorescent assay using mouse monoclonal antibody specific to HSV-1 and HSV-2 (NOVO cratra Laboratory Ltd, UK) following with second antibody FITC-conjugated $(Fab')_2$ fragment of goat anti-mouse Ig (DAKO A/S). Finally they were counterstained with Evan's Blue. Determination of the results was done by observing under fluorescence microscopy.

## Results

Among those 1,125 samples, 326 (29.97%) were cultured positive in Vero cells and 228 of 326 samples (69.34%) had enough sample for further typing. Only 207of 326 isolates (90.79%) could be successfully typed. The history of each specimen, including patient's nationality, gender and site of infection, was retrospectively reviewed (Table 1). Out of 207, 150 samples (72.46%) were from Thai patients and 57 samples (27.54%) were from foreigners. Among Thai citizen, there were 14 male and 136 female which 27 and 123 samples were collected from non-genital and genital lesions, respectively. Another group of the foreigner, all 57 samples were collected from genital area from 7 male and 50 female. Almost all genital samples were collected from female (Thai 119/123, 96.75%; Foreigner 50/57, 87.72%).

The prevalence of non-genital HSV-1 infection from 27 samples was 81.84% while that of non-genital HSV-2 infection was only 14.82%. One sample (3.70%) with mixed infection of HSV-1 and HSV-2 was found. For genital specimens, the prevalence of HSV-1 and HSV-2 infection in Thais were 39.02% and 43.09% and that of the foreigners were 36.84% and 49.12%, respectively (Table 1). Moreover, mixed infection of both HSV-1 and HSV-2 was demonstrated (Thais, 17.89% and foreigners 14.04%). There were 7 patients who had 2 samples collected from different either sites or time (Table 2). Out of 7, 3 patients (No.4, 6 and 7) had samples collected on the same day but different lesion sites.

**Table 1.** Historical background of 207 clinical specimens used in this study

| Group of patients | Site of infection* | Gender | HSV-1 | HSV-2 | HSV-1 & 2 | Total (%) |
|---|---|---|---|---|---|---|
| Thai | NG | Male | 8 | 2 | 0 | 10 (37.04) |
| | | Female | 14 | 2 | 1 | 17 (62.96) |
| | | Total (%) | 22 (81.48) | 4 (14.82) | 1 (3.70) | 27 (100) |
| | | | 23 (85.19)** | 5 (18.51)** | | |
| | G | Male | 4 | 0 | 0 | 4 (3.25) |
| | | Female | 44 | 53 | 22 | 119 (96.75) |
| | | Total (%) | 48 (39.02) | 53 (43.09) | 22 (17.89) | 123 (100) |
| Foreigner | | | 70 (56.91)** | 75 (60.98)** | | |
| | G | Male | 3 | 3 | 1 | 7 (12.28) |
| | | Female | 18 | 25 | 7 | 50 (87.72) |
| | | Total (%) | 21 (36.84) | 28 (49.12) | 8 (14.04) | 57 (100) |
| | | | 29 (50.88)** | 36 (63.16)** | | |

* NG: non-genital area, G: genital area

** Include mixed infection

**Table 2.** Results of 7 patients from two difference sites

| Patient No. | HSV-typing result (Site of collection) Sample no.1 | Sample no.2 | Distance of collection | Site of infection |
|---|---|---|---|---|
| 1 | HSV-2 | HSV-1 | 13 months | G |
| 2 | HSV-1 | HSV-2 | 7 days | G |
| 3 | HSV-2 | HSV-1 | 45 days | G |
| 4 | HSV-1 (tongue) | HSV-1 (mouth) | Same day | NG |
| 5 | HSV-1 | HSV-1 | 3 days | G |
| 6 | HSV-2 (cervix) | HSV-2 (vagina) | Same day | G |
| 7 | HSV-2 (labia) | HSV-1 (vagina) | Same day | G |

Only patient No.7 showed different HSV type. The other 4 patients (No.1, 2, 3 and 5) with different time collection, 3 patients (No.1, 2 and 3) had different type of results. All discrepant results were samples from genital area.

## Discussion

From this present study, HSV-1 seems still to be the major cause of non-genital infection among Thai patients (81.84%) although this figure may not be correct due to small sample size. In contrast to genital infection, eventhough the prevalence of HSV-2 infection was still greater than that of HSV-1 in Thai patients (60.98%), a number of genital HSV-1 infection of Thai patients (56.91%) was much increased comparing to the previous reports, 1.6-18.7% [17-19]. Similar figure of the prevalence of HSV-1 genital infection in foreigners was also observed (HSV-2; 63.16%, HSV-1; 50.88%).

High prevalence of genital HSV-1 infection had been found in many countries worldwide since early the year 1970. A history of orogenital sexual contact was more frequently reported in such case of HSV-1 genital herpes which does not exclude the possibility of genital-genital transmission. Therefore, it is believed that the changes in sexual behavior have led to an increase in the incidence of HSV-1 genital infection. This finding has to come into consideration when an intervention method to control STD in Thailand is to come into account and orogenital sex is probably more frequently practiced as a consequence of safe sex programs in the HIV campaign[10].

Moreover, the authors found mixed infection of both HSV-1 and HSV-2 in non-genital as well as genital lesions (Table 2). This phenomenon could be found as common[20]. This occurrence might be possibly caused by either superinfection with different HSV type (No 1, 2, 3) or the ability of 2 types to colonize and reactivate in the same time at anatomic region in human (No.7).

In conclusion, the authors here establish new prevalence of HSV-1 and HSV-2 infection in Thai people. It indicates that genital HSV-1 infection is now commonly found in Bangkok, the capital of Thailand similar to other countries. The increasing of genital HSV-1 infection as well as mixed infection was possibly due to the change in sexual behavior.

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## *การเพิ่มขึ้นของการติดเชื้อเฮอร์ปีส์ซิมเพล็กซ์ไวรัสไทป์ 1 และการติดเชื้อร่วมกันของเฮอร์ปีส์ซิมเพล็กซ์ไวรัสไทป์ 1และไทป์ 2 ที่อวัยวะสืบพันธุ์ ในกรุงเทพ ประเทศไทย*

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*ในระหว่างเดือนมกราคม พ.ศ.2541 ถึง เดือนธันวาคม พ.ศ. 2547, ตัวอย่างจำนวน 207 ตัวอย่างจาก 1,125 ตัวอย่างที่ส่งตรวจสามารถเพาะแยกเชื้อและจำแนกไทป์เฮอร์ปีส์ซิมเพล็กซ์ไวรัส พบว่าตัวอย่างเก็บมาจากผู้ป่วย 2 กลุ่ม คือคนไทยและคนต่างชาติ และแบ่งตามตำแหน่งการติดเชื้อ ได้เป็น บริเวณผิวหนังทั่วไป และบริเวณอวัยวะสืบพันธุ์ ความชุกของการติดเชื้อเฮอร์ปีส์ซิมเพล็กซ์ไวรัสไทป์ 1 ที่บริเวณผิวหนังทั่วไปพบร้อยละ 81.84 จากจำนวน 27 ตัวอย่าง ในจำนวนตัวอย่าง 180 ตัวอย่างที่เก็บจากบริเวณอวัยวะสืบพันธุ์ 123 ตัวอย่างของคนไทยพบมีที่ติดเชื้อ เฮอร์ปีส์ซิมเพล็กซ์ไวรัสไทป์ 1 ร้อยละ 39.02 และติดเชื้อเฮอร์ปีส์ซิมเพล็กซ์ไวรัสไทป์ 2 ร้อยละ 43.09 ในกลุ่มคนต่างชาติที่มี 57 ตัวอย่าง คือพบเฮอร์ปีส์ซิมเพล็กซ์ไวรัสไทป์ 1 ร้อยละ 36.84 และ ร้อยละ 49.12 เป็นเฮอร์ปีส์ซิมเพล็กซ์ไวรัสไทป์ 2 การติดเชื้อไวรัสทั้งสองชนิด (ไทป์ 1 และ 2) ในกลุ่มคนไทยและคนต่างชาติพบร้อยละ 17.89 และ 14.04 ตามลำดับ ความชุกของการติดเชื้อเฮอร์ปีส์ซิมเพล็กซ์ไวรัสไทป์ 1 ที่บริเวณอวัยะสืบพันธุ์ ในคนไทยพบมีการเพิ่มขึ้นตามระยะเวลาที่ผ่านมาจากร้อยละ 1.6 เป็นร้อยละ 56.91 ระหว่างปี พ.ศ. 2528 ถึงปี พ.ศ. 2547 การเพิ่มขึ้นของการติดเชื้อเฮอร์ปีส์ซิมเพล็กซ์ไวรัสไทป์ 1 และการติดเชื้อร่วมกันทั้งสองไทป์น่าจะมาจาก การเปลี่ยนแปลงพฤติกรรมทางเพศในยุคเอดส์*