CHAPTER VI

DISCUSSION

HSV can infect squamous epithelium both skin and mucosa and develops latent infection within the nerve ganglia. Infection with these viruses is common and causes a wide range of clinical syndrome. Although HSV infections in normal host are usually self-limited, patients with impaired immune systems may suffer chronic, debilitating and even fatal infection (150). In the early 1980's ACV first became available for the treatment of HSV. It has efficacy in suppressing the HSV (21). As the clinical utility of ACV became apparent, ACV is widely used. ACV is recognized as safe and effective treatments for the management of HSV infections in immunocompetent and immunocompromised populations (116,151).

For many years, HSV diseases have been successfully treated with ACV (152). Unfortunately, with increasing use of ACV, resistance has been emerged (4-6). After long term treatment of patients with ACV, the emergence of drug-resistant virus variants has been observed, and there has been an increasing number of ACV treatment failures which were associated with ACV-resistant viruses in these patient, especially in immunocompromised hosts (30). The screening of ACV^r HSV has become increasing important for choosing the appropriate therapy.

In Thailand, ACV is also used for treatment HSV infected patients. Therefore we interested to determine the sensitivity of HSV isolates to ACV by PRA. Clinical HSV isolates were collected during January 1998 – June 2002. They were totally by 121 swabs from suspected first episode and recurrent herpetic lesions at labia, eye, skin and genital lesion. These specimens were HSV culture positive by SVC. Among those, 86 isolates were successfully propagated (Table 4). The failure of propagation might be due to long term stage of specimens. Although, all of them were kept at -70^0 c, the virus might be lost, especially in the specimen with small amount of virus. All 86 isolates were mainly from female (81.40%; 70/86). Moreover, they were mostly collected from suspected genital hepetic lesion (74.42%; 64/86).

HSV typing of clinical isolates has been studied by mean of indirect IFA using MAb HSV type specific. Interestingly, in genital lesion, more than half of genital herpes was caused by HSV-1 (53.12%; 34/64) while HSV-2 were found 43.75% (28/64). These results suggested a population of individuals with a high incidence of genital HSV-1. The predominance of HSV-1 in genital infection has been variably reported worldwide as between 4-60% (112,153-162).

In Thailand, Yoosook, *et al*, in 1989, reported HSV-1 isolated from female patients genital lesion represented 1.6% of the total isolates obtained from September 1985 to April 1986 (163). The current reported by Puthavathana, *et al*, in 1991, reported that a prevalence rate of 2.6% of HSV-1 infection in genital lesions in cases collected from October 1986 to March 1991 (164). In 1998, Puthavathana *et al*, reported that a prevalence of HSV-1 infection of 18.7% in genital lesion in cases collected during the period between April 1994 and February 1996 (165).

Our results showed a prevalence of HSV-1 infection in genital lesion was 53.12%. That indicated a trend of genital HSV-1 infection was increasing in Thailand. Unfortunately, record on the history of primary or recurrent episodes of genital herpes in our subjects could not be obtained. Nevertheless, an increase in prevalence of HSV-1 associated with genital herpes is clearly shown. The results of typing indicated a change of HSV-1 epidemiology. A history of orogenital sexual contact was more frequently reported in case of HSV-1 genital herpes which does not exclude the possibility of genital-genital transmission. For these reasons, it is believed that the changes in sexual practices have led to an increase in the incidence of genital HSV-1 infection. This finding has to come into consideration when an intervention method to control STD in Thailand is to come into account and the orogenital sex is probably more frequently practiced as a consequence of safer sex programs in the HIV campaign (158).

We found that genital specimens were from female more than those from male. A possible explanation could be the different anatomy in male and female. Besides exposing a larger area, the female genital mucosa/skin may be more susceptible to HSV, both HSV-1 and HSV-2, compared with karatinised penile epithelium (158,166).

Mixed infection, infection with both HSV-1 and HSV-2, could be found only in genital lesion (3.12%: 2/64). It was possibly caused by superinfection with different HSV types, the ability of the two HSV types to colonize and reactivate in the same anatomic region in humans. This phenomenon could be found as common (167).

The susceptibility of HSV isolates to ACV was done by PRA. Only 80 HSV isolates were assayed ACV susceptibility. Two isolates with mixed infection were excluded. The other four isolates lost during propagation to get high titer of viruses. The PRA measures the overall sensitivity of virus population and equates that determination to an IC_{50}. The ACV susceptibility of HSV isolates was exhibited a wide spectrum from the range of IC_{50} both types, were 0.07-1.66 μg/ml.

When the IC_{50} value of HSV to ACV was divided according to the type (HSV-1, and HSV-2), the range of IC_{50} of HSV-1 isolates was 0.07-0.97 μg/ml and that of HSV-2 isolates was 0.17-1.66 μg/ml. It seems that, HSV-1 isolates were more susceptible to ACV than HSV-2 isolates.

The mean IC_{50} of HSV-1 isolates, 0.36 (SD=0.23), and that of HSV-2 isolates, 0.54 (SD=0.36), were statistically significant difference (p= 0.02). Our results were similar to the previous study of Yoosook *et al*, in 1989, who reported the results of ACV sensitivity of HSV isolates, the mean IC_{50}value range from 0.044-0.162 μg/ml for HSV-1 isolates and 0.008-0.504 μg/ml for all HSV-2 isolates except our range of IC_{50} of both type were wide and higher (163). In 2000, the report of Lipipun *et al* showed that antiviral activity of ACV against HSV-2 isolates with the effective dose 50% value (ED_{50}) in range 0.38-0.87 μg/ml, the mean ± SD was 0.585 ± 0.1 μg/ml (168).

Together with our results, this may suggest the increasing of ACV resistance in both types of HSV isolates. However, the measurement of the sensitivity of HSV strains to ACV was standardized by chosen threshold values for ACV-resistance. The cut off value of 3 μg/ml has been used to discriminate between ACV-sensitive and ACV-resistance isolates (36,127). According to this criteria, no ACV^r HSV was detected in this study.

To validate our system, standard HSV-1 (KOS) and HSV-2 (Baylor 186) were run in each time of assay. The mean IC_{50} of HSV-1 (KOS) and HSV-2 (Baylor 186) were 0.48 (SD=0.11) and 0.58 (SD=0.04), respectively. These IC_{50} values were nearly the same as the previous reported of Parris and Harrington, for standard HSV-1 strain KOS, is 0.4 μg/ml (130) and Kost *et al*, for standard HSV-2 strain Baylor 186, is 0.6 μg/ml (36).

The majority of ACV^r HSV has been reported to occur essentially in immunocompromised hosts, such as those infect with HIV or receiving transplants undergoing prolonged course of acyclovir chemotherapy (27,30,144,169,172). In contrast, the recovery of ACV^r HSV from immunocompetent hosts has been uncommon, and median sensitivities of HSV strains isolated before and after therapy have no significant difference (139). In our studies, all clinical HSV specimens were obtained from immunocompetent hosts thus, ACV^r HSV may rarely be detected since the prevalence of ACV^r HSV in immunocompetent hosts was previously reported only 0-7% (106,107,109). Unfortunately, no clinical samples from immunocompromised hosts were obtained in this study. However, our results could be predicted that in Thailand, ACV remains to be a drug of choice which is very safe and effective treatment to immunocompetent patients.