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The Latency Rate of Human Herpesvirus 6 (HHV6) in Positive and Negative Human Immunodeficiency Virus (HIV) Infection of Intravenous Drug Users (IVDU)

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SUMMARY **The seropositive and latency rates of HHV6 among IVDU with positive and negative HIV and control group were demonstrated. By immunofluorescent antibody test, no differences in the seropositive rates were found among these three groups. All groups had seropositive rate at the average 89% and GMT antibody 1:26. This meant that most of them had previous infection with HHV6. In addition, HHV6-DNA was determined and classified into subgroups: HHV6A and HHV6B, by polymerase chain reaction. The prevalence of HHV6-DNA indicated HHV6 latency *in vivo*. High latency rate of HHV6 was found in all three groups (the average 54%). Moreover, HHV6B (49%) had a higher frequency than HHV6A (5%); HHV6A was found only in IVDU with or without HIV infection. The result suggested that the HHV6 latency in IVDU with positive HIV may possibly transactivate HIV. The pathogenesis of HHV6 in AIDS patients should be further investigated. However, this research finding is useful for treatment, health care, prevention and control of AIDS in case of dual infections and latency of herpesvirus infections in AIDS.**

In 1986, a new herpesvius, now designated as human herpesvirus 6 (HHV6) was initially isolated from two AIDS patients with lymphoproliferative disorders, and IVDU.[5,11] After the first report, several other HHV6 isolates were found in HIV infected patients.[1, 3, 13] In 1988, the virus was recognized as the causative agent of exanthem subitum.[17] It is now beleived that HHV6 persists in the host after primary infection, can be reactivated later, and persists in the body. By characterization of isolates obtained world wide from various diseases, HHV6 is now classified into two variants, HHV6A and HHV6B.[12] Since the frequency of HHV6 isolates in AIDS patients or HIV infected individuals has been increasing rapidly, several attempts have been made to determine its destructive effect on the immune system. Therefore, this study aimed to investigate the difference in seropositive rate or latency rate of HHV6 among IVDUs with positive and negative HIV infection and normal group. The results may lead to clarification of the association between HHV6 and HIV infection.

MATERIALS AND METHODS

Sample collection

One hundred blood samples from intravenous drug users (IVDU) were collected from Thanyarak Narcotics Hospital. Another group of 53 blood samples from normal individuals as a control group were collected from donors at Siriraj Hospital, all were men with average age of 31 years. Five mililitres of each blood collection were kept in 0.5 ml 3.8% sodium citrate. Citrated blood samples were separated into plasma and blood cells by centrifigation at 2,000 x *g* for 15 minutes at 4°C. The plasma was kept at -20°C for

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virological study. The pellets of blood cells were mixed with 8 ml of lysis buffer (0.32 M sucrose, 10 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1% TritonX-100), then centrifuged at 2,000 x *g* for 10 minutes. After 2-3 times washing with lysis buffer, the clear pellets of PBMCs were obtained. The proteins of PBMCs were destroyed when incubated with 80 µl of proteinase K solution (20 mg/dl PK, 1% Tween-20, 1% NP-40, 1 x PCR buffer) overnight, at 56°C. After incubation, the mixture was inactivated at 98°C for 10 minutes and then centrifuged at 2,000 x *g* for 2 minutes. The suspensions were harvested for detection of HIV-1 proviral DNA and HHV6-DNA by polymerase chain reaction.

Polymerasc chain reaction (PCR) assay

Detection of HIV-1 proviral DNA

A nested PCR protocol for detection of HIV-1 proviral DNA[14] was adopted using 2 pairs of primers derived form pol. region of HIV-1. The sequences of the outer pair were 5'-TACAGGAGCAGATGATACAG-3' and 5'-CCTGGCTTTAATTTTACTGG -3' and those of the inner pair were 5'-GGAAACCAAAAATGATAGGG-3' and 5'-ATTATGTTGACAGGTGTAGG-3'. Briefly, treated samples (5 µl each) were placed in 50 µl total volume of a reaction mixture consisting of 50 mM KCl, 10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.01% gelatin, 100 µM each dNTP, 0.2 µM of each primer and 2 units of *Taq* DNA polymerase. The reaction underwent 30 amplification cycles at 94°C for 1 minute for DNA denaturation, 55°C for 1 minute for annealing and 72°C for 1 minute for sequence extension. The outer pair of primers was initially used in the first round of PCR. After completion, 2 µl of the first round of the reaction mixture was used as the template for the second round of PCR. The conditions were the same as the first PCR except that the inner pair of primers were used. The amplified product was detected by electrophoresis in a 1.5% agarose gel followed by UV fluorescence after staining with ethidium bromide. A molecular weight marker was also included in each gel.[15] A band of 130 base pairs was seen in the amplified products of HIV-1 proviral DNA.

Detection of HHV6-DNA

A nested PCR was used for detection of HHV6-DNA.[16] Two pairs of primers were derived from the immediate early gene locus of HHV6A, U1102 strain 10 and HHV-6B, HST strain.[16] The sequence of the outer pairs were 5'-TTCTCCAGATGTGCCAGGGAAATCC-3' and 5'-CATCATCATTGTTATCGCTTTCACTCTC-3', and those of the inner pairs were 5'-AGTGACAGATCTGGGCGGCCCTAATAACTT-3'and 5'-AGGTGCTGAGTGATCAGTTTCATAACCAA-3'. Briefly, treated samples (10 µl each) were placed in 50 µl total volume of a reaction mixture consisting of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 100 µM each dNTP, 1.25 unit of *Taq* polymerase and the outer pair of primers at concentration of 0.4 µM each. The reaction underwent 30 amplification cycles at 94°C for 1 minute for denaturation, 62°C for 2 mintues. for annealing, and 72°C for 3 mintues for sequence extension.

After completion, 5 µl of the first round reaction mixture was used as the template of the second round of PCR. The conditions were similar to the first PCR except that the inner pair of primers were used. After gel electrophoresis, a band of 184 or 413 base pairs (bp) were seen in the amplified products of HHV6A and HHV6B, respectively.

Virological study

Separated plasma of all samples were determined for antibodies against HHV6 and HIV. The antibody against HHV6 was determined by immunofluorescent antibody (IFA) testing as described previously.[17] The titer was expressed as the highest dilution yielding detectable immunofluorescence. The antibody against HIV was firstly determined by enzyme-linked immunosorbent assay (ELISA) and then confirmed with gel particle agglutination (GPA). The positive plasma by the two tests were recorded as positive HIV infected individual.

RESULTS

Blood specimens were collected from men. A total of 153 blood samples was divided into three groups: 53 from normal persons, age average 32 years, with negative HIV infection 50 from the IVDU, age average 30, with positive HIV infection and another 50 from the IVDU, age average 29.7 negative for HIV infection. Those individuals whose blood samples were antibody positive against HIV and HIV-DNA positive were recognized as positive HIV infected cases. As shown in Figure 1A, the PCR products of HIV-DNA were appeared at 130 bp in samples no. 1, 2, 3, 4, 6 and in the positive control.

Three groups of blood samples were further used to determine antibodies against HHV6 and HHV-6-DNA. The result showed that the

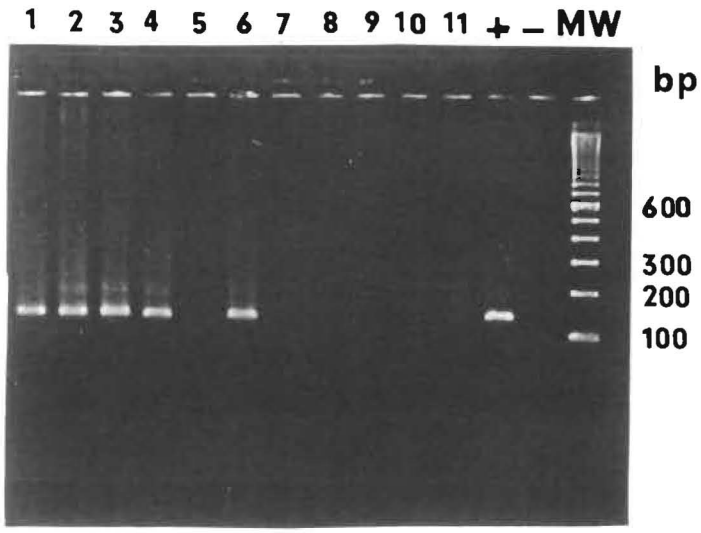

Fig. 1A PCR products of HIV- proviral DNA at 130 bp in positive control and sample nos. 1, 2, 3, 4, 6.

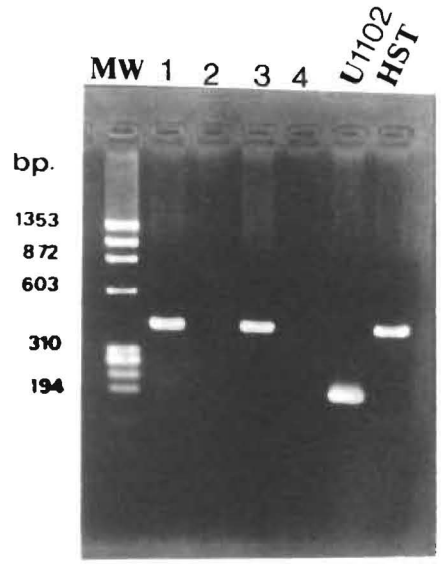

Fig. 1B PCR products of HHV6A-DNA at 184 bp in U1102 reference strain and HHV6B-DNA at 413 bp in HST reference strain and sample nos. 1 and 3.

seropositive rate and the geometric mean titer (GMT) of an antibody against HHV6 among these three groups were 96% (GMT 1:48), 90% (GMT 1:20) and 81% (GMT 1:10) in IVDU with positive HIV, IVDU with negative HIV and normal group, respectively (Table 1). These results suggested that there was not much difference in the prevalence of antibody against HHV6 among these three groups. But the GMT of HHV-6 antibody (1:48) in IVDU positive for HIV was higher than the GMT of the other two groups (GMT 1:20 and 1:10 in IVDU with negative HIV and normal group, respectively).

In addition, HHV6-DNA was also determined from PBMCs of blood samples in these three groups by PCR technique. From Figure 1B, the PCR products of HHV6-DNA could be identified into subgroups: HHV6A at 184 bp in U1102 reference strain and HHV6B at 413 bp in the HST reference strain and the samples no. 2 and 4. The results revealed that all detectable cases of HHV6-DNA were from the individuals who were antibody positive to HHV6. In comparison of HHV6-DNA among each studied group, there was similarity in the prevalence of HHV6-DNA among these three groups: 50%, 54% and 56.6% in IVDU with positive HIV, IVDU negative for HIV and the normal group, respectively. Moreover, HHV6A and HHV6B subgroups were both detected. but HHV6B (51%) was more prevalent than HHV6A (4.6%) in all studied groups, while HHV6A was found only in IVDU positive and negative HIV infected cases.

DISCUSSION

We selected IVDU as the study group because it was the high risk

Table 1. Summary of antibodies and DNAs against HIV and HHV6 in IVDU and normal groups.

Studied	Average	HHV6 Ab		HHV6-DNA*			
group	age(yrs)	positive rate	GMT	HHV6A	HHV6B	HHV6A&B	Total
IVDU (HIV+)	32	48/50 (96%)	47.5	2/50 (4%)	21/50 (42%)	2/50 (4%)	25/50 (50%)
IVDU (HIV-)	30	45/50 (90%)	19.8	2/50 (4%)	24/50 (48%)	1/50 (2%)	27/50 (54%)
Normal (HIV-)	29.7	43/53 (81%)	10.78	0/53 (0%)	30/53 (56.6%)	0/53 (0%)	30/53 (56.6%)
Total	31	136/153 (88.9%)	26.03	4/153 (2.6%)	75/153 (49.0%)	3/153 (2.0%)	82/153 (53.6%)

HHV6-DNA* = $\frac{\text{number of positive}}{\text{number of tested}}$ (%)

for blood transmission of some infectious agents such as HIV and herpesviruses groups. The seropositive rate and latency rate of HHV6 among IVDU with positive and negative HIV and the normal group in this study was the approach used to study the association between HHV6 and HIV infection in human. It was found that there was not much difference in seropositive rate among these three studied groups. The seropositive rates of all groups were about 89% which was similar to the same age group from other reports.[2,7] Alternatively, high prevalence of seropositive rate could be explained by the idea that they had been already infected with HHV6 in the early period of their life and still remained antibody positive against HHV6 after those intections.[6] The low GMT of antibody in every studied group even in the case of IVDU positive for HIV may indicate no reactivation while the GMT of antibody > 1:160 was recorded as a reactivated infection.[8] Since, serological study had been disappointing in helping to define the role of HHV6 in HIV infection, the discovery of HHV6 persistent in macrophages/monocytes indicated HHV6 latency *in vivo* by the measurement of HHV6-DNA.[6] Therefore, the next step had to be done to compare the difference of latency rate in these three studied groups. The result showed that no difference of latency rate in all groups and a higher rate of HHV-6B than HHV6A. Moreover, HHV-6A was found only in IVDU with both HIV positive and negative cases. It may be parallel to the appearance of HHV6A isolation in IVDU with symptomatic HIV infection.[5] This study suggested that HHV6A might be transmitted through blood transfusion. According to the finding of HIV and HHV6-DNAs in PBMCs, it may be per a dual infection that HHV6-DNA can be transactivated HIV and convert a latent HIV to active infection acting synergistically to enhance the viral activity of HIV.[4, 9] Therefore, this study is a preliminary one to show the latency of HHV6 and dual infection of HHV6 and HIV in IVDUs. Further investigation should be done to understand the mechanisms of the pathogenesis of AIDS. However, this study is useful for treatment and control of AIDS in case of the dual infections.

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