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Full Length Research paper

Human herpes viral central nervous system infection in human immunodeficiency virus (HIV) and non-HIV patients: An 18-month prospective study

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Human herpes virus (HHV) infection of the central nervous system (CNS) is a common problem worldwide. The incidence of HHV-CNS infection in human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/AIDS) patients and non-HIV patients has been studied at Rajavithi Hospital, Bangkok, Thailand. To identify the prevalence and incidence of HHV1 (HSV), HHV3 (VZV), HHV4 (EBV), HHV5 (CMV), HHV6A,B, and HHV 7-CNS infection, and to differentiate the clinical manifestations, laboratory findings between HSV-encephalitis and non-HSV/HHV-CNS infection amongst patients at Rajavithi Hospital, Bangkok, Thailand. An 18-month prospective study of patients with clinically suspected CNS infection was enrolled. Cerebrospinal fluid (CSF) examination and culture, along with real time polymerase chain reaction (RT-PCR) for HSV-1, HHV3, HHV4, HHV5, HHV6, HHV7 and *Mycobacterium tuberculosis* were performed. Criteria for diagnosis of HHV-CNS infection included fever, headache, seizure, alteration of consciousness, neurological localizing signs and/or neck stiffness. A total of 94 patients, 52 male and 42 female, aged between 16 - 77 years (mean + SD = 42.3 + 14.5) were enrolled between July 2008 - December 2009. Forty four patients were confirmed to be HIV/AIDS positive. Of this, 27% were treated with highly active antiretroviral treatment (HAART). There was significant difference of age and gender between the HIV/AIDS subgroup versus the non-HIV subgroup ($p < 0.026$). The incidence of HHV encephalitis was 11.3% per-year. The incidence of HHV1 (HSV) viral encephalitis, HHV5 (CMV) latent infection, and HHV4 (EBV) encephalitis accounted for 5.67, 4.2 and 0.6% per year, respectively. There were no HHV6A, B, HHV7- CNS infections observed. The incidence of HHV encephalitis was noticeably higher in HIV/AIDS patients ($p = 0.002$). The CSF /blood sugar ratio observed in HSV1 encephalitis was higher than in non HSV/HHV-CNS infected patients ($p = 0.06$). Human herpes virus, especially HSV-CNS infection was found to be common in both HIV/AIDS and non HIV patients. The incidence of VZV, EBV, HHV6 and HHV7-CNS infection were rare. Unlike the CSF/blood sugar ratio and CSF pleocytosis, clinical manifestations may not be helpful for differentiation between HSV encephalitis and non HSV/HHV-CNS infection.

Key words: Human herpes virus (HHV), central nervous system (CNS) infection, human immunodeficiency virus (HIV), viral encephalitis, herpes simplex virus (HSV), varicella zoster virus (VZV), Ebstein barr virus (EBV).

INTRODUCTION

Human herpes virus (HHV) infection of the central nervous system (CNS) is a common problem worldwide and has severe sequels unless proper treatment is implemented (Boriskin et al., 2004).

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In Thailand, to date, HHV-CNS infection in HIV/AIDS and non HIV patients has rarely been reported (Subsai et al., 2004, 2006; Windy et al., 2008). In a study from the USA (New York), HHV1 (Herpes simplex virus, HSV) and HHV3 (Varicella-zoster virus)-CNS infection were found to be 15.3 and 5.8%, respectively (Huang et al., 1999). In immunocompromised hosts, there is an increased incidence of encephalitis caused by CMV, EBV and HHV-6

Table 1. Sequences of primers used and RT-PCR product size in this study 1.

Virus	Sequence 5'-3' forward primer	Sequence 5'-3' reverse primer	PCR product (size; base pair)
HSV-1	TCCTTCTTGTCGTCTCTCTTCC	ACCATAGGATGAACAAACCACC	361
VZV	GACGGCCATTTTAGTATCAAGC	GAGGAGTGCCAATGTTACATGA	708
EBV	TGTGAACCTTATGGAGATGTGC	TATTGACCAAGCATTTCCAGTG	444
CMV	ACGATGAGTTTTCTCCGTTT	CAATAGGCTTGGTTTTCAAAGG	526
HHV 6A	GTCTAGAACTCCACCACGATCC	AGGGGAGACGAAACATAGTCAA	299
HHV 6B	TGCTAATGACATAACAGTCCC	CTCTAAACCCCGAACAGATGTC	744
HHV 7	TGTAGAATTGGCAATGTTTTCG	TTTCTCCACTAAAACAGCCCAT	732

HHV-6 infection (Windy et al., 2008). In Chiang Mai University Hospital, Thailand, the incidence rate of HHV5 (Cytomegalovirus, CMV)-CNS infection in AIDS documented cases was reported in 7 per 100 person-years in 2001 - 2002 (Subsai et al., 2004).

Clinical diagnosis of HHV-CNS infection includes: 1) fever, headache, seizure, alteration of consciousness, neurological localizing sign and/or neck stiffness; 2) performance of lumbar puncture which sows CSF lymphocytic pleocytosis, except in HHV5 (CMV) - CNS infection, which may have neutrophilic pleocytosis; 3) Increased CSF protein6 and 4) Polymerase chain reaction positivity for DNA of HHV (Table 5)

The objectives of this study were to identify the prevalence and incidence of HHV1 (HSV), HHV3 (VZV), HHV4 (EBV), HHV5 (CMV), HHV6A, B and HHV 7-CNS infection, and also to differentiate the clinical manifestations and laboratory findings between HSV-encephalitis and non-HSV/HHV-CNS infection at Rajavithi Hospital in Bangkok, Thailand.

Patients and methods

An 18-month prospective study of patients with clinically suspected CNS infection was enrolled. Cerebrospinal fluid (CSF) examination and culture, along with polymerase chain reaction (PCR) for HSV-1, HHV3, HHV4, HHV5, HHV6, HHV7 and *Mycobacterium tuberculosis*, were performed. Criteria for diagnosis of HHV-CNS infection included fever, headache, seizure, altered consciousness and neurological localizing signs +/- neck stiffness.

Patients suspected with CNS infection without contraindication for lumbar puncture, aged more than 15 years, who were able to give informed consent by themselves or a relative and able to have a lumbar puncture were enrolled. Patients suspected of having severe virulent viral infection such as bird flu (H5N1) and severe acute respiratory distress syndrome (SARS) were excluded from enrolment into this study. Clinical laboratory data included complete blood count (CBC), anti HIV results, as well as CD4 counts were recorded. Real time polymerase chain (20 µl) reaction (RT-PCR) by Sybergreen ®-Roche® used primer specific sequences for HSV-1, HHV3, HHV4, HHV5, HHV6 and HHV7, each designed to a viral target on the basis of a full search of the GenBank database (www.ncbi.nlm.nih.gov) (Boriskin et al., 2004) (Table 1) with sensitivity of 93 and specificity 100. The negative predictive value was 83% and positive predictive value 100%. *Mycobacterium tuberculosis* was also used. The total volume of CSF obtained from the patients suspected of having CNS infection, was 200 µl and underwent automated DNA extraction (MagNa pure Compact Nucleic and Isolation Kit –Roche®). The DNA was eluted into 50 µl of nuclease-free water and stored at -20°C for repeated RT-PCR analysis.

Statistics

Descriptive statistics was used for reporting the demographic data. Analytical statistics compared the mean and standard deviation (SD) in HIV and non HIV subgroups using a student-t test (Cytel Studio®). Mean incidences with 95% confidence intervals (Poisson variable) were calculated by STATA Version 6.0™. Nonparametric two independent binomial statistical analyses was done using Fisher's exact test (CYTEL®studio) for numerical data, such as number of patients. A significant p-value was $p \leq 0.05$ (two tailed).

RESULTS

A total of 94 patients, 52 male and 42 female, aged between 16 to 77 years (Mean + SD = 42.3 + 14.5) were enrolled between July 2008 - December 2009. Forty four patients were documented HIV positive, with 27% being treated with highly active antiretroviral treatment (HAART). There was significant difference of age and gender between the HIV/AIDS subgroup versus the non-HIV subgroup ($p < 0.026$). The demographics are detailed in Table 2. A total of 141 CSF samples were collected during the enrolment period.

There were 65 patients diagnosed with definitive CNS infection and 29 patients diagnosed with non-CNS infection (Figure 1). Clinical characteristics of patients with suspected CNS infection are displayed in Table 2. Non-HHV-CNS infection composed of tuberculous meningitis (n = 26), cryptococcal meningitis (n = 23) and bacterial meningitis (n = 4). Clinical characteristics and CSF findings of HHV-CNS infection are outlined in Tables 2 and 3.

The incidence of HHV encephalitis was 11.3% per year. The incidence of HHV1 (HSV) viral encephalitis, HHV5 (CMV) latent infection and HHV4 (EBV) encephalitis account for 5.67, 4.2 and 0.6% per year, respectively. There were no HHV6A, B and HHV7- CNS infections observed in the enrolled group. The incidence of HHV

Table 2. Demographic data and clinical characteristics of total patients and patients with HHV-CNS infection.

Total number	**N = 94**	
Age mean (year) ± SD, range (year)	42.3 ± 14.5 (16 - 77)	
Gender (Female : Male)	42 : 52	
Non HIV, n (%)	50(53)	
Age* mean (year)± SD, range (year)	46.2 ± 17.4 (16 - 77)	
Gender$ (Female : Male)	27: 23	
HIV, n (%)	44 (47)	
Age* mean (year) ± SD, range (year)	37.9 ± 9.2 (24 - 70)	
Gender $ (Female : Male)	15: 29	
Mean CD4 (cells per mm3.) ±SD	81.3 ±14.7	
HARRT therapy, n (%)	12 (27%)	
Sign and symptom	**Total n (%) (n=94)**	**HHV-CNS infection, n (%) (n=16)**
Headache	58 (61.7)	10 (62.5)
Fever	53 (56.3)	12 (75.0)
Stiffness of neck	46 (48.9)	13 (81.2)
Alteration of conscious	40 (42.3)	8 (50.0)
Seizure	17 (18.0)	5 (31.2)

*p=0.006 and p=0.025.

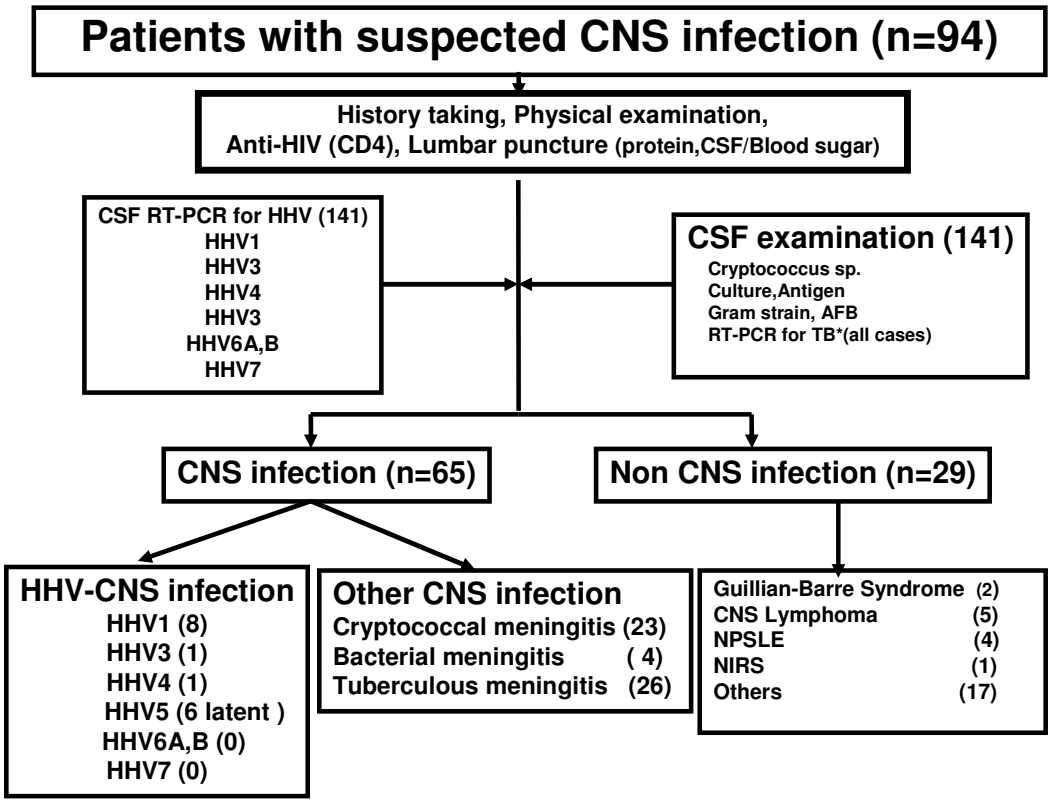

Figure 1. Diagnosis of patients with clinically suspected CNS infection in this study.

encephalitis was observed to be higher in HIV patients (p = 0.002). The CSF /blood sugar ratio (Table 6) in HSV1 encephalitis was borderline significantly higher than in patients with non HHV-CNS infection (p = 0.06).

Table 3. CSF findings in HHV-CNS infection (n=16).

Laboratory	Mean (SD)
CSF profile	
Open pressure	18.8 (3.8)
Protein	181.0 (53.7)
CSF/Blood sugar ratio	48.5 (4.7)
WBC	96.5 (49.0)
Neutrophil	7.8 (1.3)
Lymphocyte	49.8 (14.3)

Table 4. Prevalence of HHV-CNS infection, n (% prevalence).

HHV subtype	Total (n=94)	HIV (n=44)	Non HIV (n=50)
		%	
HSV1	8 (8.5)	5 (11.0)	3 (6)
VZV	47 (61)	1(2.0)*	-
CMV	6 (6)	6 (13)	-
EBV	1 (1)	1 (2)	-
HHV6A	-	-	-
HHV6B	-	-	-
HHV7	-	-	-

Table 5. Clinical characteristics of patient with HHV1 and non HHV1 infection.

Sign and symptom	HHV1 (%)	Non HHV1 (%)	p value
Fever	87.5	62.5	0.56
Headache	62.5	62.5	1.00
Seizure	37.5	25.0	1.00
Alteration of consciousness	50.0	50.0	1.00
Stiffness of neck	75.0	87.5	1.00
Focal neurological deficit	62.5	75.0	1.00

Table 6. Laboratory of patients with HSV and non HSV/HHV-CNS infection.

Laboratory CSF profile	HHV1 (n=8) Mean (SD)	Non HHV1 (n=8) Mean (SD)	p value
Open pressure	20.0 (12.4)	24.6 (17.3)	0.56
Protein	163.7 (107.9)	257.0 (295.5)	0.42
CSF/blood sugar ratio	35.5 (15.0)	19.7 (14.7)	0.06
WBC	47.8 (75.6)	204.2 (209.5)	0.19
Neutrophil	10.8 (16.5)	15.3 (24.2)	0.68
Lymphocyte	58.0 (42.7)	71.0 (36.0)	0.53
Blood CD4	15.6 (14.3)	4.3 (6.0)	0.85

There were 16 patients with HHV-CNS infection, 8 patients with HSV-1, 6 patients with CMV latent infection (without CMV retinitis), 1 patient with VZV, and 1 patient with EBV encephalitis. HSV-DNA was detected in 5 HIV positive patients (11.0%) and 3 non-HIV infected patients (6.0%) (Table 4). VZV-DNA and EBV-DNA were detected in one HIV/AIDS patient (2.0%) with clinical of VZV and EBV encephalitis. HHV6A, HHV6B and HHV7 - DNA were not detected by RT-PCR. CSF findings are shown in Table 7.

Table 7. Non HHV-CNS infection.

Diagnosis	Total (n=94)	HIV (n=44)	Non HIV (n=50)
Cryptococcal meningitis, n (%)	23 (24.4)	22 (50.0)	1 (2.0)
Tuberculous meningitis, n (%)	26 (27.6)	13 (29.5)	13 (26)
Bacterial meningitis, n (%)	4 (4.2)	1 (2.2)	3 (6.0)
Other, n (%)	29 (30.8)	6 (13.6)	23 (46.0)

DISCUSSION

This study showed that HSV was the most common cause of HHV-CNS infection (11.3%), especially in HIV positive individuals (62.5%) compared to non HIV infected patients (37.5%). The prevalence of HHV-CNS infection in Rajavithi Hospital was not different to that which has been reported in other regions worldwide (laser et al., 2003), (Ali et al., 2005; Behnam et al., 2007; Mendoza et al., 2007). This prospective study showed slightly lower prevalence of HHV-CNS infection when compared to the USA (New York) study.

This study also showed a significant increase in prevalence of HHV-CNS infection amongst HIV infected patients (p = 0.002). In contrast, in previous publication of encephalitis in Thai children (Chokephaibulkit et. al., 2001), HHV6 and HHV7 CNS infection was not identified in our enrolled adult population. Moreover, our study found that there was a significant increase in prevalence of HHV - CNS infection in HIV infected patients (p = 0.002).

In contrast with a German encephalitis study (Ali et al., 2005), we did not find seizure to be a predictive clue for HSV encephalitis when compared to non-HSV/HHV - CNS infection. In resource limited settings where RT - PCR is not often available, CSF/blood sugar ratio in HSV encephalitis seemed to be higher than non HSV/HHV - CNS infection (p = 0.06). The CSF pleocytosis (> 200 cells/mm^3) may be due to non HSV/HHV - CNS infection (p=0.19) (Table 7). However, in resource limited settings, other common CNS infection must be ruled out before diagnosis of HHV - CNS infection is established (Table 7).

Routine treatment with intravenous Acyclovir in HIV infected patients where encephalitis is suspected may be useful for up to two thirds of these patients. However, HIV infected patients with low CSF/blood sugar ratio (20%) and CSF pleocytosis greater than 200 cells/mm^3 may not receive any benefit from intravenous Acyclovir treatment, because non HSV/HHV - CNS infection was found to be the most likely cause. The most common manifestations of CMV infection in HIV patients were retinitis and CMV encephalitis was found to be rare (Chokephaibulkit et al., 2001). In this study, we found only CMV latent infection. CSF analysis including culture, RT-PCR for HHV - DNA detection and RT - PCR TB may also be useful for diagnosis of patients with neurological immune restoration syndrome (n = 1 from 94 patients in this study) (Subsai et al., 2006).

Limitations of our study was the small sample size, CSF positive controls for HHV2 were unavailable, and our study did not test for ribonucleic acid (RNA) viral infections of the CNS.

For future studies, multiplex RT - PCR using hybridization probes should be further developed, which would allow for increased sensitivity and confirmation of diagnosis.

Conclusion

Human herpes viral infections, especially HSV - CNS infection was common in both HIV positive and non HIV patients at Rajavithi Hospital, Thailand. In this study, clinical manifestations were not helpful for differentiation between HSV-encephalitis and non-HSV/HHV - CNS infection, however, CSF/blood sugar ratio (p=0.06) and CSF pleocytosis (p=0.19) were useful and may be of use in other settings, where HSV/HHV - CNS infection may be a consideration.

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