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The effect of highly active antiretroviral therapy on outcome of central nervous system herpesviruses infection in Cuban human immunodeficiency virus-infected individuals

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With the rapid progress in the development of highly active antiretroviral therapy (HAART), the observed patterns in human immunodeficiency virus (HIV) encephalitis has changed, allowing herpesvirus (HV) infection to be controlled. HAART was first administered to HIV patients in Cuba in 2001. Consequently with the aim of investigate the behavior of the HVs causing neurological disorders in this population in the post-HAART era, the authors perform a clinical evaluation by a multiplex nested polymerase chain reaction (PCR) assay for simultaneous detection of human HVs—herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), and Epstein-Barr virus (EBV). The authors studied 241 samples of cerebrospinal fluid (CSF) received at the Sexually Transmitted Diseases Laboratory between 2001 and 2005 inclusive. Of the 241 CSF studied, 10.4% resulted positive for HV infections. Of these, 92% of patients were acquired immunodeficiency syndrome (AIDS) individuals at the C3 stage. CMV (44%), EBV (28%), and dual-HV (16%) infections were the most important agents identified. The principal clinical manifestations were fever, headache, vomiting, and focal abnormalities; the latter being associated with an increased risk of death. A statistically significant result was observed when central nervous system (CNS) disease evolution was compared between patients who were under HAART against those who were not, before they developed encephalitis. It was therefore concluded that it is more likely that HIV individuals receiving HAART have a better recovery of CNS infections than those who are not receiving it. *Journal of NeuroVirology* (2007) **13,** 446–451.

Keywords: AIDS; Cuban; encephalitis; herpesviruses; HIV

Introduction

Viral infections constitute the main cause of meningoencephalitis worldwide, although other organisms can produce infection in the central nervous systems (CNS) (Steiner *et al*, 2005). Most members of the *Herpesviridae* family have been associated with sporadic cases of encephalitis in many countries, causing a wide spectrum of clinical manifestations because of the CNS tropism of these viruses (Lee *et al*, 2003; Dewhurst, 2004; Fica *et al*, 2005). Individuals with an impairment of the immune system have an increased susceptibility to herpesvirus (HV) infections. Human immunodeficiency virus (HIV)-infected individuals had been found to be at a higher risk of developing CNS HV infections because of decreased cellular immunity due to HIV infection (Kennedy, 2005). Since the introduction of highly active antiretroviral

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therapy (HAART), the incidence of some opportunistic infections has declined in some Western countries and the occurrence of HV infections declines too (d' Arminio *et al*, 2000). Moreover, due to some issues of adherence, resistance, failure, and cross-resistance, there are many patients still at risk of developing opportunistic infections (Yazdanpanah *et al*, 2001).

Cuba has the lowest HIV prevalence (0.05%) in the Americas and it is one of the lowest in the world (Pérez *et al*, 2004). The etiology of HVs was implicated by Kourí and colleagues in 2000 as agents involved in CNS infections in Cuban HIV-infected individuals after the introduction of a multiplex polymerase chain reaction (PCR) assay for HV detection in the cerebrospinal fluid (CSF) (Kourí *et al*, 2000). HAART was first administered to HIV patients in Cuba in 2001 when locally produced antiretroviral drugs started being introduced as a form of treatment (Pérez *et al*, 2004). At this time we decided to study CSF samples of HIV-infected individuals, receiving samples at the Sexually Transmitted Diseases Laboratory at "Pedro Kourí" Institute (IPK) between 2001 and 2005 inclusive, in order to understand the behavior of the HVs acting as causative pathogens in the CNS that were causing damage in the post-HAART era.

Results

A total of 241 HIV individuals were studied but only 25 (10.4%) had a positive result for HVs multiplex nested PCR assay. The mean age of these 25 individuals was 31.7 years old (age range: 23 to 45). Of these 19 were white (76%), two were mulattos (8%), and four were black (16%). Seventy-six percent of them were males. This demographic data coincide with the characteristics of the Cuban HIV population.

A CD4 lymphocyte count obtained close to the time of presentation with neurological symptoms was available for 24 patients. The median CD4 count was 137 cells/mm^3 (range: 10 to 463 cells/mm^3). According to the clinical 1992 AIDS definition case (CDC, 1993), 92% of patients suffering CNS HV infections were AIDS individuals at the C3 stage; only 37.5% of them showed a CD4 count over 200 cells. A statistical significant association between CD4 cell counts under 200 cells and deceased patients was found ($P < .05$).

Viral load was available for 10 of the 25 patients with a mean value of 125,243 (range: 530 to 740,000), 70% of them with amounts over 55,000 copies. Seventy-five percent of patients were receiving HAART before the outcome of neurological symptoms. Only three of these patients (12%) were noncompliant to treatment.

Of the 241 CSF studied, 10.4% resulted positive for HV infections and cytomegalovirus (CMV) infection was detected in 44% of the CSF samples studied. Epstein-Barr virus (EBV) was responsible for 28% of cases, PCR-positive results for human herpesvirus 6 (HHV-6) and herpes simplex virus (HSV) were obtained in 8% and 4%, respectively. Mixed infections with more than one member of the *Herpesviridae* family were present in 16% of individuals, one patient had a dual infection of CMV and EBV and the others (three patients) were infected with HSV and CMV (Table 1).

Table 1 Herpes virus detection in the CSF of HIV-infected individuals suffering of encephalitis

Infections	*Frequencies*	*%*
CMV	11	44
EBV	7	28
HHV-6	2	8
HSV-1 and -2	1	4
Mixture infections	4	16
Total	25	100

CMV: cytomegalovirus; EBV: Epstein-Barr virus; HHV-6: human herpes virus 6; HSV-1 and -2: herpes simplex viruses 1 and 2.

Five of the 25 individuals with CNS HV infections (20%) also had a positive *Cryptococcus* latex detection in their CSF; two of these were positive for CMV and the other two for EBV. In one case there was a mixed infection of HSV and CMV.

The main clinical manifestations of CNS disease in HIV-infected individuals were fever, headache, vomiting, and focal abnormalities (Table 2); the latter being associated with an increased risk of death (risk ratio [RR] = 3; 1–11) (Table 3). Furthermore, we observed a statistically significant result ($P < .05$, odds ratio [OR] = 10 [1.2–81]) when we compared CNS disease evolution between patients that were under HAART treatment against those who were not, before they developed encephalitis (Table 4). No statistical association was found between a definitive HV diagnosis and the risk of death. There was no apparent association either between the virological diagnosis and *Cryptococcus* coinfection, even with precise clinical manifestations and their relation with specific HV.

Discussion

Human HVs have been identified as an important cause of CNS disease in HIV infected individuals.

Table 2 Clinical manifestations of AIDS patients with herpesvirus-positive PCR results

	Frequencies	*%*
Fever	21	84
Headache	17	68
Vomiting	16	64
Focal abnormalities	7	28
Photophobia	5	20
Coma	3	12
Seizures	3	12
Meningeal signs	2	8

Table 3 Relationship between focal abnormalities and patients' condition at discharge

Focal abnormalities	*State at discharge*		*Total*
	Death	*Recovered*	
Yes	4	3	7
No	3	15	18
Total	7	18	25

They may act as cofactors in HIV disease progression by potentially interacting with HIV (Sivayathorn and Whitley, 1996). With the advent of PCR the scenario has changed, providing physicians with information on specific etiological agents in less than 24 h. Unfortunately, validation of the sensitivity and specificity of CSF PCR by comparison to a "gold standard," such as a brain biopsy, is only rarely available (Read and Kurt, 1999; DeBiasi and Tyler, 2004). It has also increased patients' possibilities of receiving specific and opportune antiviral drugs with a significant recovery and without long-term neuropathological manifestations (Skoldenberg, 1996; McGrath *et al*, 1997). Neurological disease has been reported as the first manifestation of AIDS in 10% to 20% of symptomatic HIV-1 infections, in which HVs have been recognized as an important cause of CNS infection (Mamidi *et al*, 2002). In 2000, Quereda and colleagues demonstrated 34% of CNS HV infections in HIV-infected individuals (Quereda *et al*, 2000); however, there are differences in incidence percentages reported by other authors (Broccolo *et al*, 2000; Minjolle *et al*, 2002).

A previous report from Kourí and colleagues in 2000 established a 22.5% of HVs infection in 182 CSF samples of Cuban HIV-infected individuals over a 3-year period (1996 to 1998) (Kourí *et al*, 2000). It has now been found that the percentage of HVs detected by PCR decreased to 10.4%. We carried out an annual distribution of HV detection in CSF of HIV patients before and after the introduction of HAART (Figure 1) taking previous data published by Kourí and colleagues into consideration (Kourí *et al*, 2000). In 2001 there was a lower diagnosis percentage (1.7%). This decrease we believe had no relation to the introduction of HAART therapy because it was not sustained throughout this period (2001 to 2005) (Figure 1).

Table 4 Relationship between patients under HAART or not and patients' condition at discharge

Under HAART	*State at discharge*		*Total*
	Death	*Recovered*	
No	4	2	6
Yes	3	15	18
Total	7	17	24*

*Data not available for 1 of the 25 patients.

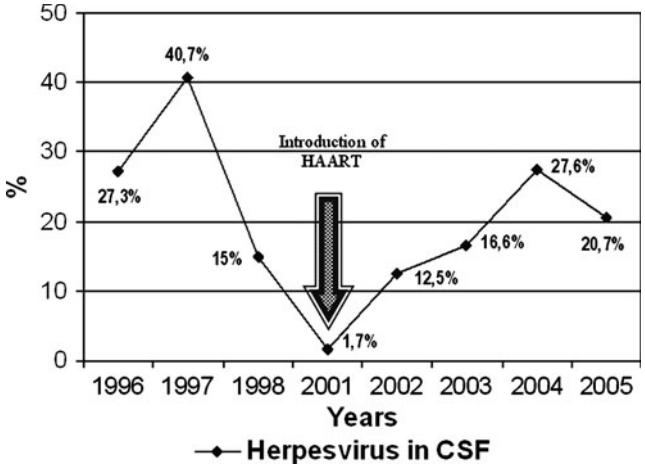

Figure 1 Percentage of Herpes virus detection by PCR in CSF of Cuban HIV-infected individuals per year, before and after HAART introduction.

Perhaps this was a coincidence because the number of patients who received HAART at that time was below 400 and this was increased over time after its introduction until 2003 when the cover of therapy was possible for 100% of patients as a previous report by Pérez and colleagues in 2004 illustrated (Pérez *et al*, 2004). The incidence behavior in following years varied; rising until 2004 when 27.6% of CNS HV detection was identified, showing that there was no dramatic reduction of HV incidence causing CNS disease. However, the influence of HAART in CNS HV infection has to be evaluated in prospective studies.

Maschke and colleagues did not find a significant change in CMV encephalitis incidence when they compared two different groups of antiretroviral-treated and nontreated HIV individual groups (Maschke *et al*, 2000), in contrast with others groups who have published data (Brodt *et al*, 1997; Michaels *et al*, 1998; Palella *et al*, 1998; Neuenburg *et al*, 2002). There are some recent reports (Gerna *et al*, 2001; Keane *et al*, 2004) that point to the transitory effect of HAART producing an inadequate long-term protection against CMV disease in patients who were severely immunodeficient prior to the treatment, in whom the immune responses against CMV declines after 3 to 5 years of treatment. Furthermore, French and colleagues in 2004 described the immnuopathological effect of immunorestoration after HAART initiation causing diseases (immune restoration disease [IRD]) uncovering HVs between the most common pathogens associated with infectious IRD (French *et al*, 2004).

The positive impact of HAART in Cuban individuals studied was found in patient survival, demonstrating that HIV individuals under HAART had a better recovery from CNS infections than those who were not receiving it (Table 3), according to other studies (Griffiths, 2004; Bernstein *et al*, 2006).

The incidence of specific HV infections in the CNS varied from one place to another; CMV incidence

seems to be predominant in individuals with HIV infection who go on to develop AIDS (Quereda *et al*, 2000; Tselis and Lavi, 2000; Deayton and Griffiths, 2000), often in those with a very low $CD4^+$ T-lymphocytes count (under 50 to 100 cells/μl) (McGrath *et al*, 1997). In concordance with other authors, CMV appears to be the virus most frequently identified in our study (44%) (Broccolo *et al*, 2000; Minjolle *et al*, 2002). EBV, HSV, and HHV-6 infections were detected in HIV-infected individuals, identifying them as etiological causes of CNS damage, with differing percentages between studied cohorts (Tang *et al*, 1997; Kourí *et al*, 2000; Quereda *et al*, 2000; Corti *et al*, 2004). Varicella-zoster virus (VZV) infection was not identified in the CSF of studied samples, but which had been reported by Toledo and colleagues in 2004 as a rare opportunistic infection occurring in 0.1% to 4% of AIDS patients with neurological diseases (Toledo *et al*, 2004).

Dual or mixed infections of the CNS of HIV populations have been described by other authors. Detection of more than one member of the *Herpesviridae* family, HVs plus other viral pathogen (JC virus [JCV]) and HVs with others nonviral pathogens (*Cryptococcus neoformans*, *Toxoplasma gondii)* in the CSF of patients with cerebral damage has been implicated in the pathogenesis of the CNS disease (Vago *et al*, 1996; Weinberg *et al*, 2005). It had also been supported by detailed autopsies of AIDS adults, which allowed the identification of a possible pathogenetic interaction of viral and nonviral infections in the CNS that could be neither clinically suspected nor diagnosed premortem (Zelman and Mossakowski, 1998; Eza *et al*, 2006).

HV infections have been estimated to be clinically indistinguishable in AIDS patients, with no specific clinical signs and symptoms and even with no characteristic changes in the CSF to support the specific etiological diagnosis (Chaudhuri and Kennedy, 2002; Boivin, 2004). However, according to the results obtained, we think that the presence of focal abnormalities could be associated with an increased risk of death among the HIV-infected individuals (Table 3).

In conclusion, our study provides substantial evidence that HVs continue to be important pathogens involved in the CNS diseases in Cuban HIV-infected individuals, in which the use of HAART does not exclude them as possible agents implicated in patients morbidity, although it is more likely that HIV individuals under HAART had a better recovery of the CNS infection than those who were not receiving it. Nonetheless, the influence of HAART in CNS HV infections needs to be evaluated in further studies. Multiplex nested PCR assay from CSF has proven to be a very useful diagnostic tool for CNS disease diagnosis, allowing the identification of etiological HVs as a single or dual infection when it is present.

Methods

Sampling and patients
The Institute of Tropical Medicine Pedro Kourí (IPK) is the only center in Cuba in charge of performing the diagnosis of herpesviral infection by PCR. Two hundred and forty-one CSF from HIV-infected individuals were received at the Sexually Transmitted Diseases Laboratory between 2001 and 2005 inclusive. All of them with signs and symptoms of central or peripheral nervous system disorders according to the criteria published elsewhere (Glaser *et al*, 2003), in which the initial suspected clinical diagnosis was HV infection, although further determinations was indicated to exclude others etiologies. The clinical files of patients with a positive PCR result for HVs were consulted retrospectively, and a list of clinical signs was recorded. We carried out an annual distribution of HV detection in CSF of HIV patients before and after the introduction of HAART (Figure 1), taking previous data published by Kourí and colleagues (Kourí *et al*, 2000).

Samples
A total of 241 CSF specimens were obtained under sterile conditions and stored at -70°C until the multiplex nested PCR assay was performed.

DNA extraction
A 39.5-μl CSF sample was used for specimen DNA extraction using proteinase K digestion as previously described (Tenorio *et al*, 1993). A 5-μl aliquot of each extraction product was used for the initial reaction.

Multiplex herpesvirus PCR assay
Different regions within the DNA polymerase genes of HVs were used for the multiplex amplification, using oligonucleotides primers reported from Tenorio and colleagues (Tenorio *et al*, 1993). The initial reaction included two mixtures of nondegenerated oligonucleotides designed, which aligned the 3′ ends with one consensus region, obtaining a product of 194 bp (base pairs). A 2-μl aliquot from the first reaction were used for the second reaction. It contained a similar mixture of 3′ homologous oligonucleotides that aligned with another consensus region (antisense 2) and a second mixture of nonhomologous and type-specific oligonucleotides selected from different regions of their aligned genomes (sense 2). The PCR mixture contained 10 mM of PCR buffer, 25 mM $MgCl_2$, 25 mM deoxynucleoside triphosphates, 5 pmol of each primer (forward and reverse), and 2 U of Taq polymerase (Invitrogen).

Routinely, 30 cycles of amplification were carried out in the following order: denaturation at 94°C for 1 min, primer annealing at 53°C (first reaction) and subsequently at 47°C (nested PCR) for 1 min, followed by primer extension at 72°C for 1 min. The reaction products were run on 4% agarose gel and DNA bands were stained with ethidium bromide. Different size

amplification products from each human HV were detected depending on their size, HSV (120 bp), VZV (98 bp), CMV (78 bp), HHV-6 (66 bp), EBV (54 bp), while pseudorabies virus (PRV) was used as a internal control (140 bp).

In order to avoid contamination, risks were eliminated by separating work areas between mixture preparations. In the first and nested reaction different, RNase- and DNase-free pipettes and filter tips were used; 5 μl of DNase- and Rnase-free water, used as negative and positive controls of HSV, CMV, and EBV, were included too.

Statistical analysis

Descriptive SPSS 11.5 statistical package was used to process all the data.

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