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Laboratory studies

Low prevalence of human herpesvirus-6 and varicella zoster virus in blood of multiple sclerosis patients, irrespective of inflammatory status or disease progression

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ABSTRACT

Herpesviruses, including human herpesvirus-6 and varicella zoster virus, have been implicated in the disease aetiology of multiple sclerosis. These viruses are capable of reactivation, reminiscent of the relapsing-remitting nature of multiple sclerosis. However, viral DNA has also been reported present in healthy controls, often at similar prevalence rates. This study aimed to determine whether prevalence could be associated with different stages of activity of the disease as well as the inflammatory status of the patients. Polymerase chain reaction assays were used to screen for human herpesvirus-6 and varicella zoster virus DNA in blood from 31 Caucasian patients with multiple sclerosis and 30 healthy age, sex and race matched control subjects. The patients were screened for inflammation using C-reactive protein as a marker and were also categorized according to their remitting/relapsing status. Results were positive for human herpesvirus-6 in blood from only one patient (3.2%) and human herpesvirus-6 DNA was not present in any control subjects. Varicella zoster virus was not detected in either the patients or control subjects. Similar to some other studies we saw an absence or very low viral positivity in blood from both patients and controls. These findings were irrespective of relapse episodes, increased inflammatory status or duration of the disease. Results therefore do not support a causative role for either human herpesvirus-6 or varicella zoster virus in the disease aetiology of multiple sclerosis, but rather that prevalence in patients may be linked to that of the general population.

1. Introduction

The presence of exogenous viruses such as human herpesvirus-6 (HHV-6) and varicella zoster virus (VZV) has been investigated in patients with multiple sclerosis (MS) but the reported prevalence rates range from 0% to 100%. Their presence has also been reported in healthy control subjects and in patients with other neurological disorders, often at a similar prevalence to those seen in patients with MS. These viruses can establish latency in the central nervous system (CNS) or the immune system, with periodical reactivation [1,2], reminiscent of the relapsing-remitting nature of MS. HHV-6 is a ubiquitous virus, causing infection in early childhood followed by latent infection [3] and is periodically reactivated [4]. The virus also has the ability to induce demyelination [4], which is a characteristic of MS. VZV causes the highly infectious disease known as chickenpox. The virus stays in the latent form in the sensory nerves and can cause shingles when reactivated. The reactivation can be spontaneous or can be caused by a period of illness or stress [5].

Although herpesviruses have been detected in patients with MS, reports vary substantially. Ablashi et al. reported HHV-6 DNA in peripheral blood mononuclear cells (PBMC) in 67% of patients with MS in remission, but also in 60% of healthy controls [3]. Ordoñez et al. [6] reported VZV DNA in the cerebrospinal fluid (CSF) from 65% of patients with progressive (primary and secondary) MS, while Mancuso et al. [7] reported VZV DNA to be absent in the CSF of patients with MS (including relapsing-remitting MS, chronic progressive MS and clinically isolated syndrome). None of the patients in the study reported by Mancuso et al. [7] were undergoing immunosuppressive or immunomodulating therapy. Similar results have been shown for VZV in brain tissue. Sanders et al. [8] reported VZV DNA in post mortem brain tissue from 43% of patients with MS, but also in 32% of patients with other neurological diseases, while in contrast Burgoon et al. [9] found no VZV DNA in either the CSF or in acute plaques in brain tissue from patients with MS. Ordonez et al. [10] reported the prevalence of VZV was

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Table 1
Primer sequences for human herpesvirus-6 and varicella zoster virus

Reference	Virus	Gene	Primer sequences	Band size
NCBI	HHV-6	U83 F	5′-TTAGGATGTTTGTTGTGTTGC-3′	182 bp
NC_001664/000898*	A + B	U83 R	5′-TCAAGACTTGTCGGCGATGC-3′	
Sotelo et al. [13]/GenBank	VZV	ORF10 F	5′-GCTACCGGTCACATGGAACT-3′	216 bp
		ORF10 R	5′-TTCACAGACCGCGATGTAAG-3′	

bp = base pairs, HHV-6 = human herpesvirus-6, NCBI = National Center for Biotechnology Information, ORF = open reading frame, VZV = varicella zoster virus.
* Primers have been designed to detect HHV-6 Variant A and B.

Table 2
Custom gene sequences (positive controls) for human herpesvirus-6 and varicella zoster virus

Reference	Virus	Gene	Custom gene sequence	Custom gene sequence size
NCBI NC_000898 (variant B)	HHV-6	U83	5′-ATGGCTATCGGATTTATCGGTAGTTCCCCCGATGCGG AGCTGTCTTCAGAAAATTCACGTATTTCGTCTTCTGTCTTATT AGGATGTTTGTTGTGTTGCACAGATTGGTCCGCTGTCGTAC CTGGGAAGACAGAGACTTTCAGAAAACCTTTTGTTGCA ATCATGATTAAAAAGCTAAAAAGTTGTTTTGCTGCTTACCT GTCTGATTTAGAGCAGGGCTCGATGTGTGATATGGCAAA CGCATCGCCGACAAGTCTTGAATTAGGATTGTCGAAATT AGACAAAGAATCATGA-3′	294 bp
GenBank Gene ID: 1487675	VZV	ORF10	5′-GGCCCGAACTAAGAATTGCTATAAGTGAACCATCT ACATCTTTGGTGCGATATGCTACCGGTCACATGGAACTT GCCGAATGGTTTTTATTTTCACGTACACATAGTTTAAAGCC ACAATTTACCCCAACGGAACGGGAAATGTTAGCGTCA TTTTTTACGTTGTATGTTACTCTTGGTGGAGGAATGTTGAACT GGATCTGTAGAGCAACTGCAATGTATTTAGCTGCTCCTTA CCATTCCCGTTCGGCTTACATCGCGGTCTGTGAATCTCTG CCCTATTACTATATCCCGGTTAATAGTGACCTGTTATGTGA TTTAGAGGTATTACTGTTAGGCGAGGTCGACCTCCCAAC TGTTT-3′	360 bp

bp = base pairs, HHV-6 = human herpesvirus-6, NCBI = National Center for Biotechnology Information, ORF = open reading frame, VZV = varicella zoster virus.

different when they investigated different VZV genes in PBMC from patients with MS, with prevalence rates for VZV open reading frame (ORF)31, ORF62, ORF63 and ORF67 of 60%, 13%, 67% and 7%, respectively.

The aim of this study was to investigate the presence of HHV-6 and VZV DNA in peripheral blood (whole blood) from patients with MS, both in actively diseased patients and during remittance of the disease, as well as during different stages of inflammatory activation as measured by C-reactive protein (CRP), a marker for both inflammation and infection. Whole blood samples were used to include both peripheral blood leucocytes and plasma. In this regard, HHV-6 establishes latent infection in lymphocytes [11] while VZV does so in nerve ganglia [12,13] but is transient in PBMC [13].

2. Methods

2.1. Study population

Ethics approval for the study was obtained from the Health Sciences Research Ethics Committee of the Cape Peninsula University of Technology. The study was conducted in accordance with the Declaration of Helsinki (1964). The study population consisted of 31 Caucasian female patients and 30 age, sex and race matched control subjects. Patients with MS were contacted and recruited through the MS Society, Western Cape Branch, South Africa. Written informed consent was obtained from all participants. Patients were diagnosed by a neurologist based on the McDonald Diagnostic Criteria (2005 revision) which specifies that at least two different events (lesion formation in the CNS) must occur, separated in time as well as space, before diagnosis can be confirmed. Additional tests included CSF analysis and visual evoked potential recordings which were further used to confirm diagnosis [14–17]. The diagnosis of all patients recruited for this study was confirmed by a neurologist based on clinical, laboratory and, specifically, MRI findings included in the diagnosis of MS. Six of the patients were actively diseased (in relapse), 11 had a relapse 5–12 months prior to the study (intermediate stage) and 14 had not relapsed for more than a year (in remission). Clinical features experienced during active relapse episodes include loss of sensation, muscle weakness, spasticity, visual loss, in coordination, cognitive impairment, pain, and bladder and bowel disturbances [14–16]. The exclusion criteria used in this study included the use of fatty acid supplements, interferon or cortisone, or presence of a second disease for patients with MS and presence of a disease in control subjects. Ten patients were using non-steroidal anti-inflammatory drugs (NSAID) and five patients were using immunosuppressive drugs.

2.2. Polymerase chain reaction assays

A standard polymerase chain reaction (PCR) technique was used to screen for HHV-6 (using U83, a functional viral chemokine specific to HHV-6) and VZV (using ORF10) in human DNA from peripheral whole blood. Primers for the amplification of U83 (HHV-6) were designed to amplify both variants A (National Center for Biotechnology Information [NCBI] NC_001664) and B (NCBI NC_000898). Primer sequences for ORF10 (VZV) were taken from Sotelo et al. [13]. Primer sequences used in this study are summarised in Table 1. Primers were used at a final concentration of 0.5 μM and assays were optimized to include 8 mM $MgCl_2$ and 15% dimethyl sulfoxide. PCR reactions were performed using GoTaq Hot Start Polymerase (Promega, Whitehead Scientific, Cape Town, South Africa), which consisted of an initial 5 minute denaturation step at 94 °C, followed by 40 cycles consisting of a denaturation step for 30 seconds at 94 °C, an annealing step for 1 minute at 55 °C (U83) or 52 °C (ORF10) and an extension for

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