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CLINICAL REPORT

Human Herpesvirus 6 and 7 DNA in Peripheral Blood Leucocytes and Plasma in Patients with Pityriasis Rosea by Polymerase Chain Reaction: A Prospective Case Control Study

ANTONIO AN TUNG CHUH[1], SUSAN SHUI SENG CHIU[2], and JOSEPH SRIYAL MALIK PEIRIS[3]

Departments of [1]Medicine, [2]Paediatrics and [3]Microbiology, University of Hong Kong, China

An association between pityriasis rosea and human herpesvirus 7 (HHV-7) has been reported but remains controversial. The purpose of the present study was to investigate the association between HHV-6 and HHV-7 with pityriasis rosea. Fifteen patients aged 6–54 years with a diagnosis of pityriasis rosea and 15 age-matched controls were recruited. None of the patients had HHV-6 or HHV-7 DNA detected by polymerase chain reaction in the acute or convalescent plasma specimen. In the acute peripheral blood leucocytes specimen, 3 patients and one control had HHV-6 DNA detected ($p = 0.299$; NS), while 7 patients and 5 controls had HHV-7 DNA ($p = 0.355$; NS). Antibody to HHV-6 was detected in the acute specimen of 13 patients and 13 controls, while antibody to HHV-7 was found in all 15 of patients and controls. We thus found no evidence of recent HHV-6 or HHV-7 infection in patients with a diagnosis of pityriasis rosea. ***Key words: skin disease; virus; primary infection; reactivation.***

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Antonio A. T. Chuh, Department of Medicine, University of Hong Kong, Shop B5, Ning Yeung Terrace, 78 Bonham Road, Ground Floor, Hong Kong. E-mail: achuh@iohk.com

The cause of pityriasis rosea (PR) is unknown. Drago et al. (1, 2) reported the detection of human herpesvirus 7 (HHV-7) DNA by nested polymerase chain reaction (PCR) in the skin, peripheral blood leucocytes (PBL) and plasma of all of their 12 patients with PR. They failed to detect it in plasma and skin of 11 control specimens. They found HHV-7 DNA in 44% of the control PBL specimens, although the intensity of the reaction was reportedly weaker. It is not known whether the control subjects were matched for sex and age. Other investigators have failed to confirm this finding (3).

Kempf et al. (4) detected HHV-7 DNA and expression of the HHV-7-specific immunodominant pp85 antigen in 1 out of 13 lesional skin biopsy specimens of PR and in 2 out of 14 biopsy specimens of control subjects. In this retrospective study, plasma and PBL were not examined.

Kosuge et al. (5) detected HHV-7 DNA in 13 out of 30 (43%) samples of PBL of the patients with PR and 14 out of 25 (56%) samples of PBL of controls. Human herpesvirus 6 (HHV-6) DNA was detected in 6 out of 29 (21%) patients with PR and 9 out of 23 (39%) controls. Although a few cases had significant changes in antibody titres to HHV-6 or HHV-7, the greater proportion of patients had no definite increase in antibody titres.

However, the detection of HHV-6 and HHV-7 DNA in PBL only is of limited significance since viral DNA persists in the PBL of healthy persons. Viral DNA detection in a cell-free body fluid such as plasma has been shown to correlate better with active viral replication (6, 7) and is a more appropriate specimen. Furthermore, controls recruited in this study were not age or sex matched.

We report here a prospective case control study of PR patients and age- and sex-matched controls where HHV-6 and HHV-7 DNA in both plasma and PBL were investigated.

The aim of this study was to investigate the association between HHV-6 and HHV-7 with PR.

MATERIAL AND METHODS

Between 1 August 1999 and 31 January 2001, all patients with a diagnosis of PR in a primary care setting were invited to join the study. The diagnosis was based on clinical grounds.

There are no universally accepted diagnostic criteria for PR. Our diagnostic criteria were "an acute eruption of discrete circular or oval lesions with peripheral collarette scaling pattern and central clearance on some or all lesions, and with some or all lesions orienting along direction of the ribs". Neither a herald patch nor a truncal and proximal limb distribution was mandatory for our diagnosis, as atypical rash distribution is fairly common for PR. Our diagnostic criteria did not exclude atypical rash morphology including purpuric and vesiculo-bullous variants. However, lesion biopsies for histopathology were to be arranged for all atypical cases.

Blood in EDTA was collected on the day of initial presentation and a convalescent sample was collected 4 weeks later. For each patient diagnosed with PR, the next patient of the same sex and comparable age (± 2 calendar years) requiring blood collection for non-dermatological disease who consented to participate in the study was recruited as a control. Informed written consent was obtained from all study and control subjects, or from the parents or legal guardians of minors. The protocol of this study was approved by the Ethics Committee, Faculty of Medicine, University of Hong Kong (EC 1347-00).

Extraction of viral DNA from PBL and plasma and PCR for HHV-6 and HHV-7 DNA was carried out using previously described methods (6). Positive and negative controls were used in each experiment and standard precautions to prevent PCR cross-contamination were adhered to. Serology for HHV-6 and HHV-7 antibodies was done using immunofluorescent tests on virus-infected cell smears, as previously described (6). The PCR investigations were carried out blinded to the clinical status of the patient. Similarly, the serology results on the acute and convalescent patient samples and the control serum were performed in parallel but were read "blinded" to the clinical information. In addition, a complete blood count was performed on each patient.

We used Fisher's exact probability test, two-tailed, to analyse PCR results and qualitative serology data.

RESULTS

In total, 30 patients were recruited, 15 being study subjects with PR, and 15 being matched control subjects. The patients

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with PR were aged between 6 and 54 years (mean age: 26.8 years). Three were children aged 6, 9, and 11 years. Six (40%) were males and 9 (60%) were females. Apart from an English lady, aged 29, the others were Chinese. Clinical features and findings of the 3 children have been reported previously (8).

A lesion biopsy was performed in one study subject with atypical PR features, revealing focal spongiosis with perivascular lymphocytic infiltrates compatible with PR. Biopsy was not performed for the other 14 patients with typical PR.

The 15 matched control subjects ranged in age from 8 to 52 years (mean age: 27.3 years).

PCR for HHV-6 DNA was performed in acute and convalescent blood samples of all 15 patients and in the initial blood of the controls. None (0%) of the patients had HHV-6 or HHV-7 DNA detected by PCR in the acute or convalescent plasma. Three (20%) patients and one (7%) control had HHV-6 DNA detected in the acute PBL specimen ($p = 0.299$; NS). Seven (47%) patients and 5 (33%) controls had HHV-7 DNA detected in the acute PBL specimen ($p = 0.355$; NS). Antibody to HHV-6 was detected in the acute specimen of 13 (87%) patients and 13 (87%) controls, and antibody to HHV-7 was found in all 30 (100%) patients and controls.

One of the controls, an 11-year-old boy presenting with fever and acute otitis media, had evidence of a primary HHV-6 infection – HHV-6 DNA present in the acute plasma specimen in the absence of HHV-6 antibody and seroconversion demonstrated in the convalescent blood. Otitis media is in fact one of the manifestations of primary HHV-6 infection (9).

DISCUSSION

Neither HHV-6 nor HHV-7 appears to play an aetiological role in our series of 15 patients with PR. Although HHV-7 DNA was detected in PBL in 47% of patients with PR, this was not significantly different from that in the matched controls. More importantly, HHV-6 or HHV-7 viral DNA was not detectable in the plasma of any of these patients. Our results concur with those of Kempf et al. (4) and Kosuge et al. (5) suggesting lack of good evidence of active or primary HHV-7 infection in patients with PR. PCR for HHV-7 DNA in plasma was not performed in either study. PR might be associated with primary infection or reactivation of HHV-7 and detection of HHV-7 in the plasma may be useful in detecting either. In our study, we investigated HHV-6 and HHV-7 DNA in the plasma as well as in PBL and we had age- and sex-matched controls available for comparison.

Based on the detection of antibodies to the Epstein-Barr virus (EBV) early antigen, Bonafe et al. (10) reported an association between PR and EBV. They found no association with influenza A, B, parainfluenza 1, 2, 3, adenovirus, respiratory syncitial virus, *Mycoplasma pneumoniae*, ornithosis-psittacosis, Q-fever, herpes simplex virus, varicella or cytomegalovirus.

It has been demonstrated in one controlled trial that erythromycin might have been of benefit in patients with PR (11, 12). In addition to its effect on bacteria (including atypical agents), this antibiotic has anti-inflammatory and immunomodulatory effects (13) that could contribute towards its action in PR. If the latter is true, dysfunction of the immune system, in particular cellular immunity, may be important in the pathogenesis of PR. EBV infection has immunomodulatory effects and the age distribution of primary EBV infection matches that of PR. We thus consider it worthwhile that future studies investigate the roles of EBV, atypical bacteria and cellular immune dysfunction in PR.

Since PR is a transient self-limiting disease, recruiting patients in a primary care setting is important. None of our patients required hospitalization and these cases would have been missed if our study had been hospital based. Some patients with PR may be referred to a specialist dermatology department. However, because the waiting list usually exceeds 1–2 months in the local setting, the rash would usually have faded by the time these patients are seen by the dermatologist. The present study had the advantage that the primary care physician had training and certification in both paediatrics and dermatology.

In a case control study of 15 patients with PR, we failed to identify an association of HHV-6 or HHV-7 infection or reactivation in active PR. We conclude that HHV-7 is unlikely to be a significant factor in the pathogenesis of PR. We recommend further studies of PR to be along other directions including EBV, atypical bacteria and cellular immune dysfunction.

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