Prevalence and molecular characterization of human herpes virus 6 and 7 in patients with pityriasis rosea using polymerase chain reaction in a tertiary care hospital, Puducherry

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ABSTRACT

Background: Human herpes virus (HHV) 6 and 7 belongs to Betaherpesviridae a sub-family of Herpesviridae. HHV-6 and HHV-7 are double stranded DNA group viruses. The cause of PR and the disease caused by HHV is not well established. The association of HHV 6 and HHV 7 with pityriasis rosea (PR) is under controversy. The HHV DNA is also detected in normal controls which evidence the non-significance of HHV in the pathogenesis of PR.

Methods: The study design was planned for 35 consecutive cases of pityriasis rosea of all age groups. This study was carried out at the department of Dermatology and Central Research Lab, at Sri Manakula Vinayagar Medical College and Hospital, a tertiary care hospital in Puducherry. Since there is no universally accepted diagnostic protocol for PR, we have planned to detect HHV-6 and 7 DNA by PCR from the lesions of all clinically diagnosed cases of pityriasis rosea.

Results: In contrary to the expected outcome that HHV-6 and HHV-7 positivity would correlate to the possible pathogenesis of the disease, the PCR results of all the 35 samples from clinically suspected PR cases were found to be negative.

Conclusions: We conclude that HHV-6 and HHV-7 viruses may not always play a role in the pathogenesis of the disease. Thus PR speculated to have more than one etiology may have agents other than HHV6 and HHV7 involved in the pathogenesis of the disease.

Keywords: Human herpes virus, Pityriasis rosea, Polymerase chain reaction

INTRODUCTION

The study aims to reveal the association of human herpes virus (HHV) 6 and HHV-7 with pityriasis rosea (PR) by polymerase chain reaction (PCR).

The objective of the study was to isolate the HHV-6 and HHV-7 DNA from skin biopsy of the lesions and to confirm the presence of HHV-6 and HHV-7 viruses by PCR.

HHV-6 and HHV-7 belongs to Betaherpesviridae a sub-family of Herpesviridae. HHV-6 and HHV-7 are double stranded DNA group viruses that are 2000 Å and 1700 Å in size respectively. The structure of the virus is composed of an outer lipid bilayer below which is present
an icosahedral capsid consisting of 162 capsomeres. HHV-6 enters the CD 46 and HHV-7 uses CD 4+ as receptor for invasion. The virus interferes with various cellular functions, multiplies, matures in the cytoplasm and causes cell rupture. HHV-6 exists as two variants A and B, although they have almost similar genes, difference persists at viral protein level, also their growth pattern in different cell types.

The association of HHV-6 and HHV-7 with pityriasis rosea (PR) is under controversy. Pityriasis rosea is a papulosquamous skin disorder characterised oval or annular plaques with peripheral collarette of scales. The initial lesion is a herald patch followed by eruption of the rest of the lesions in inverted fir tree pattern. The cause of the disease is not well studied. Studies suggest that it could have a viral etiology. The disease usually lasts from weeks to months and disappears spontaneously. It occurs mostly in ages 3-45 years and is more prevalent in people of age 11 to 20 years followed by those 21 to 30 years. In the U.S. incidence has been calculated as 130 per 100,000 males and 140 per 100,000 females. The epidemiology of pityriasis rosea in India is not well studied.

There is no well-defined disease pattern proposed by the infection of HHV except, exanthema subitum (ES) or "roseola", caused by HHV-6 which is characterized by high temperature and skin rashes. The cause of PR and the disease caused by HHV is not well established. The HHV-7 DNA was detected in patients with PR in 1997 which evidenced the correlation of HHV with PR. The HHV DNA is also detected in normal controls which evidence the non-significance of HHV in the pathogenesis of PR. The primary focus of this proposal is to find the prevalence of the HHV-6 and HHV-7 causing PR in the rural area of Puducherry. In this study we try to establish an association between HHV-6 and HHV-7 with pityriasis rosea (PR) by polymerase chain reaction.

METHODS

This study was carried out at the department of Dermatology, Venereology and Leprosy and Central Research Lab, at Sri Manakula Vinayagar Medical College and hospital in puducherry between december 2015 to december 2016. The study design was planned for 35 consecutive cases of Pityriasis rosea of all age groups.

Since there is no universally accepted diagnostic protocol for PR, we have planned to detect HHV-6 and 7 DNA by PCR from the lesional skin of all clinically diagnosed cases of pityriasis rosea.

Sampling

Skin biopsy from the active lesions was collected from 35 consecutive clinically suspected cases of PR and DNA from all the samples was extracted by phenol chloroform method. Clinical histories including drug history recording all the symptoms and signs and other conditions associated with the presentation from onset of the disease through the course of the illness to the physician and date of skin biopsy done was documented.

Polymerase chain reaction

Primers

Three sets of primers from different regions of the HHV-6 genome will be used to amplify the HHV-6 DNA by nested polymerase chain reaction (PCR) as described by the sequences of the first set of primers designated for generating a 186-base pair (bp) fragment (primer pair 1) will be 5'-CCCATTATACGATTTCCTGCAC-3' as sense primer and 5'-TTCAAGGGACCTTTATTGTGATT-3' as antisense primer. The second set of primers was derived from a highly conserved region shown to encode the major capsid protein. In this experiment, a two-step amplification procedure will be performed to increase the specificity, as the set consisted of outer primers (primer pair 2), 5'-GGCTTTTCAGTTGTAATTCCGACG-3' and 5'-TGGCGGATCTGACAGATACGGAGG-3', codes for 520-bp fragment, and an inner pair (primer pair 2), 5'-GCTGACACTTTGTGTGCAGACG-3' and 5'-ATGCGA-AACACGTCTGTACAGT-3', which yields a fragment of 258-bp. In order to determine the variants of HHV-6 the third set of primers (primer pair 3), derived from a sequence corresponding to the immediate-early gene, will be employed. The sequences of the primers for this purpose will be 5'-TTCTCAGATGTGCAGAGGGAATG-3' and 5'-ATCGA-TGTTGACGCTCTAC-3', in generation of 325-bp and 553-bp fragments for type A and type B variant, respectively.

For the HHV-7 PCR, outer primers (P1): 5'-TAT CCC AGC TGT TTT CAT ATA GTA AC-3'; P2: 5'-GCC TTG CGG TAG CAC TAG ATT TTT TG-3') which amplify a fragment of 186 bp, and inner primers (P3: 5'-CAG AAA TGATG ACA GAT GTT GG-3'; P4: 5'-TAG ATT TTT TGA AAA AGA TTT AAT AAC-3') which amplify a fragment of 124 bp will be used and protocol will be followed.

RESULTS

Patients age group ranged from 8 to 50 years. Most patients were between the age group 20-30 years as given in (Figure 1). There was almost equal sex distribution as seen in (Figure 2). All the cases presented us within the first week of the illness. Skin biopsy specimen from the new eruptions was used for PCR study. The primer sequences for both HHV-6 and 7 viruses used for PCR is shown in (Table 1). The lesional biopsy samples of all the patients subjected to PCR with these primer sequences did not match. In contrary to the expected outcome that
HHV-6 and HHV-7 positivity would correlate to the possible pathogenesis of the disease, the PCR results of all the 35 skin biopsy specimen from clinically suspected PR cases were found to be negative.

**Table 1: Primer for polymerase chain reaction of HHV-6 and HHV-7.**

| Virus         | Primer (5'-3')                     | Product size |
|---------------|------------------------------------|--------------|
| HHV-6         | F: CCCATTACGATTTCCTGCAC R: TTCAGGGACCGTTATGTCATT | 186 bp       |
| HHV-6 outer   | F: GCGTTTTCAGTGTAGTTCGCGCACG R: TGGCCCGCATTCTGACAGATACGGAGG | 520 bp       |
| HHV-6 inner   | F: GCTAGAAACGTAATTGCGCGAGAACG R: ATCCGAAACCAACTGTCTGACTGGCA | 258 bp       |
| HHV-7 outer   | F: TATCCAGCTTTTCATATAGTAAC R: GCCTCGGTAGCAGATTTTGT | 186 bp       |
| HHV-7 inner   | F: CAGAAATGATAGACAGATGTTGG R: TAGATTTTTTGAAAAAGATTTAACA | 124 bp       |

**Figure 1: Age distribution.**

**Figure 2: Sex distribution.**

**DISCUSSION**

Pityriasis rosea is an acute self-limiting, papulosquamous disorder with very characteristic skin rash and clinical course, mainly involving children and young adults. The etiology of pityriasis rosea is unclear, but several factors suggest an infectious cause clustering of cases within families, a history of preceding upper respiratory tract infection, a primary lesion followed after an interval by a widespread typical rash and self-terminating course, recurrence is rare, suggesting that there is long-lasting immunity after infection.

HHV-6 was first isolated from patients with lymphoproliferative disorders in 1986 by Salahuddin et al and named as human B lymphotrophic virus. In 1991, the National Institutes of Health classified HHV-6 into HHV-6A and HHV-6B. HHV-6A may contribute to the pathogenesis of multiple sclerosis. HHV-6B is the major causative agent of exanthem subitum.

Some studies have demonstrated the presence of HHV-7 in lesional skin and in mononuclear cell, plasma, and peripheral blood, suggesting their role in the pathogenesis of pityriasis rosea, but others have not.

Some drugs can cause a pityriasis rosea like eruption. These include arsenic, bismuth, gold, omeprazole, metronidazole, isotretinoin, barbiturates, clonidine, captopril, imatinib and ketotifen. Pityriasis rosea like eruptions can also occur following hepatitis B and BCG vaccination.

In a typical case, the diagnosis is easy because of the characteristic morphology and distribution of lesions. The eruption persists from 3 to 8 weeks, sometimes as long as 3 to 6 months and then spontaneously clears. HHV-6 and 7 share the similar cell tropism and disease associations.

HHV-7 is highly prevalent worldwide and primary infection occurs during childhood but later than that caused by HHV-6. After primary infection, the latency is established in peripheral blood T cells.

The results of our study revealed that the HHV-6 and HHV-7 viruses although hypothesised to be etiological agents, were found to be negative for all the 35 study patients. Similar study results were found from study by Werner et al in 1999 which showed that pityriasis rosea is not associated with HHV-7. It has been made clear that both HHV-6 and HHV-7 are well adapted to their human host since even in the developed world seroprevalence rates are almost universal and they are rarely pathogenic in the immunocompetent host. Pityriasis rosea can be seen more as a reaction pattern than a specific disorder caused by a single aetiological agent.
This suggests that we have to consider agents other than HHV-6 and HHV-7 as etiology for this disease. However the sample size was less in our study, further such studies have to be done in the future with larger sample size to prove the possible etiological agents in pityriasis rosea.

Hence, we conclude that HHV-6 and HHV-7 viruses may not always play a role in the pathogenesis of the disease. Thus PR speculated to have more than one etiology may have agents other than HHV-6 and HHV-7 involved in the pathogenesis of the disease.

ACKNOWLEDGEMENTS

I acknowledge the Department of Dermatology and the Central Research lab, faculty and staff for rendering great help during the study.

Funding: Funded by Sri Manakula Vinayagar Medical College and Hospital

Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee (SMVMCH- EC/DDO/AL/762/2015)

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Cite this article as: Murugaiyan R, Kaliyaperumal K, Rangasamy B. Prevalence and molecular characterization of HHV-6 and HHV-7 in patients with pityriasis rosea using polymerase chain reaction in a tertiary care hospital, Puducherry. Int J Res Dermatol 2019;5:728-31.