

# Human Herpesvirus 6 and 7 in Pityriasis Rosea<sup>1</sup>

## PİTİRİYAZİS ROZEADA HERPES VİRUS 6 VE 7

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### Summary

**Purpose:** Pityriasis rosea is a papulosquamous skin disease of unknown origin. Human herpesviruses have been associated with pityriasis rosea, but there have been several contradictory reports on the role of human herpesvirus infections in the development of pityriasis rosea. We aimed to study further the involvement of human herpesvirus 6 and human herpesvirus 7 in the etiology of pityriasis rosea, and to review all studies reported until now concerning the relationship between human herpesvirus and pityriasis rosea.

**Materials and methods:** We performed polymerase chain reaction analysis for human herpesvirus 6 and human herpesvirus 7 in patients with pityriasis rosea. Samples of peripheral blood mononuclear cells, plasma and skin from 32 patients with pityriasis rosea were obtained. Fifteen healthy subjects and 15 patients with other skin diseases as controls were selected in the polymerase chain reaction analysis.

**Results:** Human herpesvirus 6 DNA sequences were detected in 8 of 32 (25%) skin samples of patients with pityriasis rosea, while plasma and peripheral blood mononuclear cells samples were negative. Human herpesvirus 6 DNA was found in 1 of 15 skin samples of patients with other dermatoses, but neither in their plasma nor in their peripheral blood mononuclear cells. Human herpesvirus 7 DNA was not demonstrated in skin lesions, plasma and peripheral blood mononuclear cells of any patients with pityriasis rosea. Human herpesvirus 7 DNA was detected only in the peripheral blood mononuclear cells specimen of one patient with other dermatoses, but neither in their plasma nor in their skin lesions. No human herpesvirus 6 or human herpesvirus 7 DNA were detected in the skin, plasma and peripheral blood mononuclear cells of any healthy controls.

**Conclusion:** These results, especially HHV-6 DNA positivity in primary and secondary lesions may suggest that the human herpesvirus 6 may play a part in pathogenesis of some patients with pityriasis rosea.

**Key Words:** Pityriasis rosea, Human herpesvirus 6,  
Human herpesvirus 7

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### Özet

**Amaç:** Pityriyazis rozea nedeni bilinmeyen papüloskuamöz bir hastalıktır. Hastalığın oluşumunda herpes virus 6 ve 7 etken olarak ileri sürülmüştür. Ancak pityriyazis rozea oluşumunda herpes virüslerinin rolüne ilişkin birbiri ile çelişkili bildiriler bulunmaktadır. Bu nedenle pityriyazis rozea ile herpes virus 6 ve 7'nin ilişkisini araştırmayı ve şimdiye kadar bu konuda yapılmış çalışmalarını gözden geçirmeyi amaçladık.

**Materyal ve Metod:** Otuz iki pityriyazis rozealı hastanın periferik kan mononükleer hücreleri, plasma ve deri lezyonu örneklerinde polimeraz zincir reaksiyon (PCR) yöntemi ile herpes virüs 6 ve 7'nin varlığı araştırıldı. Kontrol grubu olarak da sağlıklı 15 kişi ile başka deri hastalığı bulunan 15 hasta alındı.

**Bulgular:** Pityriyazis rozealı hastaların deri örneklerinin %25'inde (8/32) herpes virus 6 DNA dizilerine rastlanırken, plasma ve periferik kan mononükleer hücre örneklerinde herpes virüs 6 DNA dizilerine rastlanmadı. Herpes virus 6 DNA'sı başka bir deri hastalığı bulunan 15 hastanın sadece 1 deri örneğinde saptandı. Ancak onların plazma ve periferik kan mononükleer hücre örneklerinde herpes virüs 6 DNA'sı saptanmadı. Pityriyazis rozealı hastaların deri lezyonları, plazma ve mononükleer hücre örneklerinin hiçbirinde herpes virüs 7 DNA'sına rastlanmadı. Herpes virüs 7 DNA'sı başka deri hastalığı bulunan 15 kontrol hastasının sadece 1 mononükleer hücre örneğinde saptandı. Fakat bu hastaların deri ve plazma örneklerinde herpes virüs 7 DNA'sı saptanmadı. Herpes virüs 6 ve 7 DNA'sı sağlıklı kişilerin deri, plazma ve mononükleer hücre örneklerinin hiçbirinde tespit edilmedi.

**Sonuç:** Bu sonuçlar, özellikle deri lezyonlarında herpes virüs 6 DNA'sının bulunması, herpes virüs 6'nın pityriyazis rozealı bazı hastaların patogeneğinde rol aldıklarını düşündürmektedir.

**Anahtar Kelimeler:** Pityriyazis rozea, Herpes virus 6,  
Herpes virus 7

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Pityriasis rosea (PR) is a common, acute, self-limiting papulosquamous disorder. The disease is present world-wide, no racial predominance has been noted (1). About the pathogenetic processes resulting in clinical presentation of PR is hardly known. In general, three different hypothesis can be delineated: (i) PR is an inflammatory reaction of the skin to a non-infectious exogenous or endogenous agent. (ii) PR is an infectious skin disease. (iii) PR is an inflammatory multifactorial skin disease which can be induced by various infectious and non-infectious agents. Many features suggest an infectious etiology of PR. The higher prevalence during an altered state of immunity like during pregnancy or after bone marrow transplantation, low rate of recurrence as a result of a long-standing, in most cases even life-long immunity, prodromi, evolutionary sequence of skin lesions, dyskeratotic cells and multinucleated giant cells in the epidermis, elevated erythrocyte sedimentation rate, decrease of T-cells and increase of B-cells in the peripheral blood, response to pooled gammaglobulin or plasma of convalescent patients and induction of a PR-like eruption by subcutaneous injection of tissue fluid from PR lesions are features suggesting an infectious cause of PR (2). However, various common infectious agents have been excluded as the cause of PR (3,4). Recently, Drago and coworkers opened the discussion on a viral pathogen in PR by reporting the detection of human herpesvirus (HHV)-7 in the skin, plasma, and peripheral blood mononuclear cells (PBMC) specimens from PR patients (5,6). But, this association between HHV-7 and PR was not confirmed by other authors (7-9).

To further study the role of HHV-6 and HHV-7 in the pathogenesis of PR, we studied polymerase chain reaction (PCR) analysis of skin, plasma and PBMC of Turkish patients with PR. In addition, we reviewed all studies reported until now concerning the relationship between HHV and PR.

### Patients and Methods

Thirty-two patients with acute PR (18 male and 14 female) aged 16 to 43 years were included

in this study. Blood and skin samples were obtained from otherwise healthy and immunocompetent patients with PR; patients with acquired immune deficiency syndrome or receiving immunosuppressive therapy were excluded. The diagnosis of PR was made by clinical and histological examinations. Thirty-two biopsy specimens were obtained from 32 patients with acute phase of PR. The skin biopsies were taken from 20 primary and 12 secondary lesions. The median time between the onset of the disease and a skin biopsy was 12.3 days (range, 5-30 days) Specimens were divided into two pieces through longitudinal axis. One of the twin pieces was sent to histological examination. Others were saved at  $-80^{\circ}\text{C}$  in the deep freezer until PCR analysis were performed. PBMC and plasma were separated by Ficoll-Hypaque gradient centrifugation, and were saved using the above procedure.

Fifteen healthy subjects (9 male and 6 female; range 19-40 years) undergoing plastic surgery and 15 patients (8 male and 7 female; range 21-54) with other dermatoses (4 psoriasis, 3 vitiligo, 3 lichen planus, 1 ashy dermatoses, 1 allergic contact dermatitis, 1 drug eruption and 2 alopecia totalis) as controls were selected and studied. Informed consent was obtained from all subjects and all tests were performed and evaluated blindly.

### Polymerase Chain Reaction

Approximately  $10^6$  PBMC, 100  $\mu\text{l}$  of plasma and skin specimens were pretreated with pronase E and its buffer at  $40^{\circ}\text{C}$ , 60 minute, and overnight for skin specimens, and then nucleic acids were extracted with alkali-phenol and chloroform method. Extracted nucleic acids were dissolved in 200  $\mu\text{l}$  DNase and RNase free deionized distilled water and then subjected to nested PCR as described previously (6). The HHV-6 sequences of outer primer were *5'ccgcaatcgatccacctagegg3'* and *5'gtgagaacggattcgaacagtgctg3'*. Inner primers were *5'cccatttacgatttctgcaccacctc3'* and *5'ttcagggaccgttatgtcattgagcat3'*. The first round PCR, in briefly, the thermal cycler was programmed first to preheat at  $95^{\circ}\text{C}$  for 10 min to

denature samples and then samples (10µl of extracted DNA was mixed with 2,0 mM MgCl<sub>2</sub>, 30 pmol each outer primers, sense and antisense, 2 U Taq DNA polymerase, 10 mM each deoxynucleoside triphosphate) were subjected to 30 cycles consisting of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s with a thermal cycler (MJ Research). Then 5 µl of the first PCR product was amplified as described previously for 30 cycles with inner primers.

The HHV-7 sequences of outer primer were 5'-*agttccagcactgcaatcg*-3' and 5'-*cacaaaagctcgctatcaa*-3'. Inner primers were 5'-*cgcatcaccaaacctactg*-3' and 5'-*gactcattatggggatcgac*-3'. The HHV-7 DNA thermal condition included initial denaturation at 94 °C for 10 min, denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s for 30 cycles. Then 5 µl of the first PCR product was amplified as described previously for 30 cycles with inner primers.

A set of known positive and negative controls was included during all steps of the DNA isolation and amplification procedure. To avoid contamination of specimens with viral DNA, standard precautions concerning spatial separation of pre- and post PCR steps and aliquotation of reagents were followed strictly. To control integrity of DNA isolated from specimens and/or presence of PCR-inhibitory substances in these samples, beta-globin DNA PCR was performed in each specimens.

Nested PCR products of HHV-6 and HHV-7 (186 bp of for HHV-6 and 264 bp for HHV-7) were analyzed by ethidium bromide staining and UV transillumination following electroforetic separation (45 min, 120V) on 2% agarose gels. Standard size markers were used to identify the expected amplicon lengths of 186 and 264 bp.

### Results

In all samples, beta-globin gene sequences were amplified indicating functionality of PCR. HHV-6 DNA sequences was detected in 8 of 32 (25%) skin samples of patients with PR, including primer and secondary lesions. HHV-6 DNA was

not detected the plasma and PBMC samples of all patients with PR. HHV-6 DNA was found in 1 of 15 skin samples of patients with other dermatoses (this patient had alopecia totalis and was receiving systemic steroid for 3 months), but neither in their plasma nor in their PBMC.

HHV-7 DNA was not demonstrated in skin lesions, plasma and PBMC of any patients with PR. Only in one PBMC specimen of patients with other dermatoses (psoriasis) were HHV-7 DNA sequences detected, but neither in their plasma nor in their skin lesions. Neither HHV-6 nor HHV-7 DNA was detected in the skin, plasma and PBMC of any healthy controls.

### Discussion

In 1997, Drago et al (5,6) searched the presence of HHV-7 sequences in skin, plasma, and PBMC of 12 patients with PR, and reported all PR specimens to be HHV-7 positive by nested PCR. They detected HHV-7 sequences in PBMC of 11 healthy individuals (44%), but not in their plasma. No HHV-7 sequences were detected in the skin of any dermatologic controls. We did not detect HHV-7 DNA in skin lesions, plasma and PBMC of any patients with PR. We detected HHV-7 sequences only in one PBMC sample of dermatologic controls. They observed a faint signal of HHV-6 DNA sequences in PBMC from 6 controls (24%) and 6 patients (50%), but neither in their plasma nor in their skin. We found HHV-6 DNA in skin samples from 8 patients with PR and one patient with other skin disease as control. However, this patient was receiving immunosuppressive therapy. Moreover, they showed cytopathic effects in SupT1 cells after cocultivation with PBMC of those patients, and herpesvirus-like particles were found in the supernatants of cocultures by electron microscopy. The authors suggested that the detection of HHV-7 DNA in plasma reflects viral replication in patients with PR (5).

Watanabe et al. (8) investigated for HHV-7 DNA in plasma samples by applying the same PCR protocol as Drago et al. and detected HHV-7 sequences in only about 50% (17/36) of samples, as opposed to 100% in Drago et al.'s report. They

did not detect HHV-7 sequences in the 31 plasma samples from age- and gender- matched healthy controls. They further investigated for the prevalence of antibodies to HHV-7 by indirect immunofluorescent assay in the same plasma samples, but all cases, including healthy controls, were negative for IgM. A significant increase of IgG was detected in one PR patient; however, neither a significant increase nor a decrease in titer of IgG in the other PR patients was observed. We cannot say anything about the prevalence of antibody since we could not perform indirect immunofluorescent assay in the samples. They cannot fully confirm a direct causal relationship between PR and HHV-7 because the viral sequences were transiently found in about half of the patients and the results of indirect immunofluorescent assay did not suggest the reactivation of HHV-7 in PR.

Yoshida et al (9) also applied the same nested PCR protocol to peripheral blood DNA of PR patients and healthy subjects, and found a signal of equal intensity in all samples. This finding argues against an increase in viral DNA load in the PBMC of patients with PR and against the specific occurrence of the viral sequences in the PR samples. But, Drago et al (10) reported that the whole blood is not the same substrate as PBMC or plasma, and using it may lead to incorrect conclusions.

Yasukawa et al. (11) detected HHV-6 and HHV-7 genomes in PBMC from six (43%) and one (7%) of the 14 patients, respectively, whereas neither genome was apparently amplified from the DNA of control individuals by the PCR method. We did not detect any HHV-6 and HHV-7 genomes in PBMC from patients with PR and healthy controls. They also failed to detect any increase in anti-HHV-6 and anti-HHV-7 IgG relative to normal control subjects. They suggested that marked viral replication did not occur in these patients, and a lack of association between PR and HHV-7 infection. However they suggest that HHV-6 is reactivated in some patients with PR.

Kempf et al. (7) focused on the presence of HHV-7 in skin biopsies of herald patches and sec-

ondary PR lesions by a nested PCR protocol and immunohistochemical analysis were employed. HHV-7 was detected in only 1 of 13 (8%) archival skin biopsies of PR lesions, which represented a smaller incidence than in controls of normal skin (2 of 14 biopsies, 14%). We did not detect HHV-7 DNA in skin samples of patient with PR and control subjects. They detected the expression of HHV-7 specific antigen only in the PR skin biopsy specimens that contained the viral DNA sequences and in 1 of 2 specimens of normal skin harboring the HHV-7 DNA sequence. They concluded that the low detection rate of HHV-7 DNA sequences and antigens argues strongly against a causative role for HHV-7 in the pathogenesis of PR.

Kosuge et al. (12) detected HHV-7 DNA in 13 of 30 (43%) samples of PBMC of the patients with acute PR and 14 of 25 (56%) samples of PBMC of controls (other skin disorders). HHV-6 DNA was detected in 6 of 29 (21%) patients with acute PR and 9 of 23 (39%) controls. They reported that there was no difference in the prevalence of HHV-6 or HHV-7 in PBMC of patients with PR and those with other skin diseases. They observed that a diagnostic rise (at least fourfold) in titre of HHV-7 antibody in two patients and a decrease in titre of HHV-7 in antibody in five patients. A rise in titre of HHV-6 antibody was observed in two patients and a fourfold decrease in titre in two patients in the seroepidemiological study performed. They concluded that HHV-7 and HHV-6 may play a part in some patients with PR, but the other causative agents may exist. We detected HHV-7 only in one PBMC sample of patients with other skin diseases. We detected neither HHV-6 nor HHV-7 DNA in PBMC specimens of patients with PR and healthy controls.

Offidany et al. (13) investigated the presence of HHV-7 using the PCR in several body samples such as urine, saliva, PBMC and scales from 12 patients with active PR in parallel with 20 healthy controls. They detected HHV-7 DNA sequences only in saliva samples of five patients. PBMC and urine from patients did not demonstrate HHV-7

**Table 1.** Summary of previous PCR studies (PBMC: peripheral blood mononuclear cells; \*: Peripheral blood (see the text); Numerals show the number of patients)

Investigators	Human herpesvirus 6						Human herpesvirus 7											
	Pityriasis rosea			Control			Pityriasis rosea					Control						
	Skin	Plasma	PBMC	Skin	Plasma	PBMC	Skin	Plasma	PBMC	Urine	Saliva	Scales	Skin	Plasma	PBMC	Urine	Saliva	Scales
Drago et al.(5,6)	0/12	0/12	6/12	0/12	0/25	6/25	12/12	12/12	12/12	-	-	-	0/12	0/25	11/25	-	-	-
Yasukawa et al.(11)	-	-	6/14	-	-	0/15	-	-	1/14	-	-	-	-	-	0/15	-	-	-
Kempf et al.(7)	-	-	-	-	-	-	1/13	-	-	-	-	-	2/14	-	-	-	-	-
Watanabe et al.(8)	-	-	-	-	-	-	-	17/36	-	-	-	-	-	0/31	-	-	-	-
Kosuge et al.(12)	-	-	6/29	-	-	9/23	-	-	13/30	-	-	-	-	-	14/25	-	-	-
Offidani et al.(13)	-	-	-	-	-	-	-	-	0/12	0/12	5/12	0/12	-	-	0/20	0/20	14/20	0/20
Yoshida M.(9)	-	-	-	-	-	-	-	4/4*	-	-	-	-	-	3/3*	-	-	-	-

specific sequences, nor was HHV-7 detected in scales from lesional skin. However HHV-7 was demonstrated in saliva samples from 14 controls subjects, while urine, scale and PBMC were negative. These findings could not support the idea of a correlation between HHV-7 and PR. They could not also observe HHV-7 DNA in PBMC samples of patients with PR and healthy subjects as in our study.

As seen Table 1, the prevalence of HHV-6 and HHV-7 DNA was found different in various studies. The wide range of PCR positivity on the PBMC of reported several studies may depend on the different sensitivities of PCR and/or on the different geographical prevalence (14). Kosuge et al reported that differences in the time of sample collection might explain the different results. They speculated that more than one agent causes PR. HHV-6 is one of the other candidates (12). The spectrum of varying and contradictory data may represent the nature of PR (2).

Our findings suggest a lack of association between PR and HHV-7 infection, but Drago et al (14) reported that some HHV-7 genes may transactivate those of HHV-6 and may stimulate HHV-6 replication and reactivation. Furthermore, once reactivated, latent HHV-6 genomes may become prominent, leading to disappearance of those of HHV-7 or impairing their detection by PCR and serology. This may explain why HHV-6 may be easily isolated from PBMC after HHV-7 reactivation and also why other HHV-6 antigens can be

boosted by HHV-7 infection and may be detected in skin and PBMC (14).

In summary, it is notable that the HHV-6 DNA was detected in skin samples from 8 (25%) of 32 patients with PR, and was not detected any samples from healthy controls. In addition, for a pathogenetic association between HHV and PR, the virus is expected to be present in lesions (7), and PR is a multifactorial disease, which can be induced by various infectious and non-infectious agents (2). We conclude that HHV-6 may play a part in pathogenesis of some patients with PR.

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