

Is There a Link between Human Herpesvirus Infection and Toll-like Receptors in the Pathogenesis of Pityriasis Rosea? A Case-control Study

Mostafa Abou El-Ela¹, Eman Shaarawy¹, Mohamed El-Komy¹,
Marwa Fawzy¹, Rania Abdel Hay¹, Rehab Hegazy¹, Amin Sharobim³,
Nadine Moustafa¹, Laila Rashed², Khalda Sayed Amr⁴

Departments of ¹Dermatology and ²Clinical Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt, Departments of ³Dermatology and ⁴Molecular Genetics, National Research Center (NRC), Cairo University, Cairo, Egypt

Corresponding author:

Rania Abdel Hay, MD
13th Abrag Othman
Kournish el Maadi
Cairo 11431
Egypt
raniamounir@kasralainy.edu.eg

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ABSTRACT Human herpesvirus (HHV) 6 and 7 are involved in the pathogenesis of pityriasis rosea (PR). Our aim was to evaluate the role of the innate immune response in PR through the detection of Toll-like receptors (TLR) 2, 3, 4, 7, 8, and 9 expression in the skin of affected patients and to detect the possibility of being induced by HHV-6 and/or HHV-7 viral coexistence in these patients. Twenty-four patients with PR and 24 healthy controls were included in this case-control study. Biopsy was obtained from the PR lesion and from the healthy skin of controls for detection of HHV-6 and 7 as well as TLRs 2, 3, 4, 7, 8, and 9 gene expression using real-time polymerase chain reaction (PCR). Significantly elevated expression of all studied TLRs and significantly higher viral load of HHV-6 and 7 in PR cases were detected. A significant higher expression of TLR2 and 4 in HHV-7 positive cases and a significant positive correlation between TLR9 and HHV-7 viral load were documented. HHV6 and 7 may also be involved in the pathogenesis of PR via TLR pathways.

KEY WORDS: innate immunity; pattern recognition receptors; RT-PCR; viral coexistence; viral reactivation

INTRODUCTION

Even though pityriasis rosea (PR) has been recognized as a medical condition for many years and much has been done to describe and diagnose the rash, little is yet known regarding its etiology. There are many studies supporting the theory that PR is caused by an infectious agent (1). These studies were based on several facts, including the resemblance of its rash to viral exanthemas, the rare recurrences of PR suggesting lifelong immunity after a single episode (2), the occurrence of seasonal variation, the clustering in some communities, as well as the appearance of flu-like symptoms in some patients (3). There is evi-

dence of human herpesvirus (HHV) 6 and 7 association with PR. There is no consensus whether PR is a consequence of primary viral infection in adolescents and young adults or due to virus reactivation from a latent state (3-5).

Toll-like receptors (TLRs) are a group of pattern recognition receptors (PRRs) that are involved in mechanisms of host defense against a wide range of pathogenic microorganisms (6). TLR 3, 7, 8, and 9 are intracellular TLRs that sense virus-derived pattern molecules and respond with the induction of antiviral genes, such as type I interferon (IFN) (7). TLR 2 and 4

have been identified as signaling membrane surface receptors activated by bacterial wall components (8) and by some viral antigens as well (9). Expression of TLR 3, 7, and 9 has been previously detected in blood lymphocytes from patients with PR (10).

The aim of this study was to evaluate the role of innate immune response in PR for the first time through the detection of TLR 2, 3, 4, 7, 8, and 9 expressions in the skin of affected patients and to detect any possibility of being induced by HHV-6 and/or HHV-7 viral coexistence in these patients.

PATIENTS AND METHODS

The present case-control study was approved by the ethical research committee office (REC), Dermatology Department, Faculty of Medicine, Cairo University, and all participants signed a full informed written consent prior to participation.

This study included 24 patients with classic active PR and 24 age and sex-matched apparently healthy individuals serving as controls. All participants were recruited between May 2012 and January 2013 from the dermatology outpatient clinic, Kasr Al Ainy Hospital.

All patients had the classical findings of PR and were in the active stage of the disease (having active lesions appearing within the same week of their visit to the clinic). All participants were otherwise healthy and immunocompetent, none of them had a recent

history of immunization, and none of them were on systemic steroids or other immunosuppressive therapy. Medical history and a complete physical and dermatological examination were performed for every patient.

A 4 mm punch biopsy was obtained from the active edge of one of the lesions from each patient and from the apparently healthy skin of the trunk from each control.

The collected specimens were stored for further processing for quantitative detection of HHV-6 and 7 by real-time polymerase chain reaction (RT-PCR) using HHV-6 and 7 Real-TM Quant supplied by Sacace Biotechnologies USA: detection of TLR 2, 3, 4, 7, 8, and 9 gene expression by RT-PCR was also performed (11). The sequence of the primers used for RT-PCR in this study is summarized in Table 1.

Statistical methods

Data were statistically described in terms of mean \pm standard deviation (SD), frequencies (number of cases), and relative frequencies (%) when appropriate. Comparisons between groups were done using the T-test for normally distributed quantitative variables, and the chi-square (χ^2) test for categorical data. Correlation between various variables was done using the Pearson moment correlation equation for linear relation. $P < 0.05$ was considered statistically significant. All statistical calculations were done using the computer programs Microsoft Excel (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 19 for Microsoft Windows.

RESULTS

This case-control study included 24 patients (8 women (33.3%) and 16 men (66.7%)) with classic active PR lesions that appeared within the previous two weeks of recruitment. Their age ranged from 19-45 years (mean \pm SD 28.62 \pm 8.30). Twenty-four, apparently healthy, age and sex-matched individuals ($P=0.370$ and $P=1$ respectively) were also included in this study as controls; they were 8 women (33.3%) and 16 men (66.7%), and their age ranged from 19-46 years (mean \pm SD 30.83 \pm 8.59).

Human herpesvirus 6 and 7 results

In the current study, 20 cases (83.3%) showed positivity for HHV-6 and/or HHV-7. Of the included cases, 66.7% ($n=16$) were found to be HHV-7 positive, while 33.3% ($n=8$) of cases were HHV-7 negative. This was significantly higher in comparison to controls, where 9 individuals (37.5%) were HHV-7 positive and

Table 1. Sequence of the primers used for real-time polymerase chain reaction

	Primer sequence
TLR2	Forward: 5'- For: AACTTACTGGGAAATCCTTAC-3' Reverse: 5'- AAAAATCTCCAGCAGTAAAAT-3'
TLR3	Forward: 5'- GCATTTGTTTTCTCACTCTTT-3' Reverse: 5'-TTAGCCACTGAAAAGAAAAT-3'
TLR4	Forward: 5'- CGAGGAAGAGAAGACACCAGT-3' Reverse: 5'- CATCATCCTCACTGCTTCTGT-3'
TLR7	Forward: 5'-AAACTCCTTGGGGCTAGATG-3' Reverse: 5'-AGGGTGAGGTTCTGTGGTGT-3'
TLR8	Forward: 5'-CTGTGAGTTATGCGCCGAAGA-3' Reverse: 5'-TGGTGCTGTACATTGGGGTTG--3'
TLR9	Forward: 5'-CGCCCTGCACCCGCTGTCTCT-3' Reverse: 5'-CGGGGTGCTGCCATGGAGAAG-3'
GAPDH	Forward 5' GGATTTGGTCTATTGGG 3' Reverse 5' GGAAGATGGTGATGGGATT3'
HHV-6	Forward: TTCTCCAGATGTGCCAGGGAAATCC Reverse: CACATTGTTATCGCTTCACTCTC
HHV-7	Forward: TTGTATCCGAGCATTATGATG Reverse: CGTACACCGAGTTACATGTTG

TLR: Toll-like receptors; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; HHV: human herpesvirus

Table 2. Summary of the descriptive data of Toll-like receptors in both groups

	Patients (n=24)	Controls (n=24)	P value
TLR2 (Range, mean ± SD)	1.02-2.80 (1.97±0.49)	0.08-0.61 (0.3±0.18)	<0.001*
TLR3 (Range, mean ± SD)	5.2-12.8 (9.89±1.76)	1.9-6.3 (3.64±1.33)	<0.001*
TLR4 (Range, mean ± SD)	0.43-1.6 (0.97±0.34)	0.04-0.41 (0.19±0.09)	<0.001*
TLR7 (Range, mean ± SD)	0.83-2.4 (1.57±0.45)	0.11-0.6 (0.3±0.14)	<0.001*
TLR8 (Range, mean ± SD)	2.5-6.4 (5.13±1.3)	1.1-3.5 (1.98±0.62)	<0.001*
TLR9 (Range, mean ± SD)	0.31-1.7 (0.94±0.33)	0.12-0.49 (0.29±0.12)	<0.001*

n: number; TLR: Toll-like receptors; SD: standard deviation; * $P < 0.05$ was considered significant

the other 15 subjects (62.5%) were found to be HHV-7 negative ($P=0.043$).

Regarding the viral load among HHV-7 positive patients, the PR cases showed a significant higher viral load (range: 14567-32180, mean ± SD 22829.69±4791.44) in comparison to the HHV-7 positive controls (range: 8210-16230, mean ± SD 11159.89±2548.94) ($P < 0.001$).

On the other hand, 41.7% (n=10) of the included cases were found to be HHV-6 positive, while 58.3% (n=14) of cases were HHV-6 negative. There was no statistically significant difference in comparison to controls, as 6 of them (25%) were HHV-6 positive and 18 subjects (75%) were HHV-6 negative ($P=0.221$).

Regarding HHV-6 viral load, PR cases showed a significantly higher viral load (range: 1983-3910, mean ± SD 2713.5±603.2) in comparison to controls (range: 1010-1724, mean ± SD 1265.33±283.22) ($P < 0.001$).

Toll-like receptors expression:

A summary of the descriptive data of different TLRs in both groups is presented in Table 2. Comparing the TLR 2, 3, 4, 7, 8, and 9 expression levels be-

tween patient and control groups revealed that the mean levels of the studied TLRs were significantly higher in patients in comparison with controls (Table 2). Furthermore, when comparing the TLR 2, 3, 4, 7, 8, and 9 expression levels within the patient group, it was found that both TLR 2 and 4 were significantly higher among those who were HHV-7 positive in comparison with HHV-7 negative cases ($P < 0.001$) (Table 3).

Studying the correlations between the different studied variables in the patient group revealed a significant positive correlation between TLR2 and TLR4 expression levels ($r=0.680$, $P < 0.001$) (Figure 1). In addition, a significant positive correlation between TLR9 expression levels and HHV-7 viral load was detected ($r=0.599$, $P=0.014$) (Figure 2).

DISCUSSION

The current study serves as a further proof of the assumed role played by both the innate immunity and viral coexistence in PR. HHV might also be involved in the pathogenesis of PR via TLR pathways.

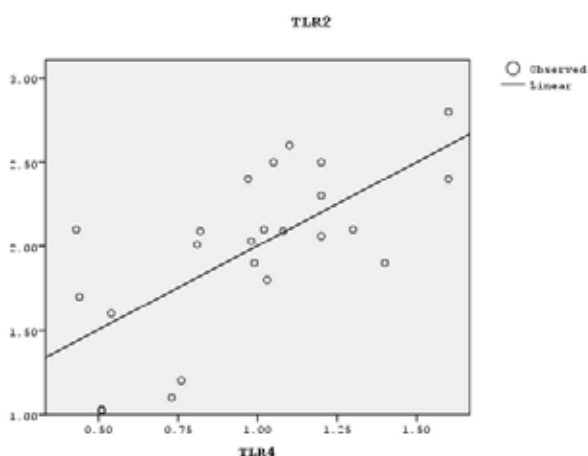


Figure 1. Positive correlation between Toll-like receptor (TLR) 2 and TLR4 expression in pityriasis rosea cases.

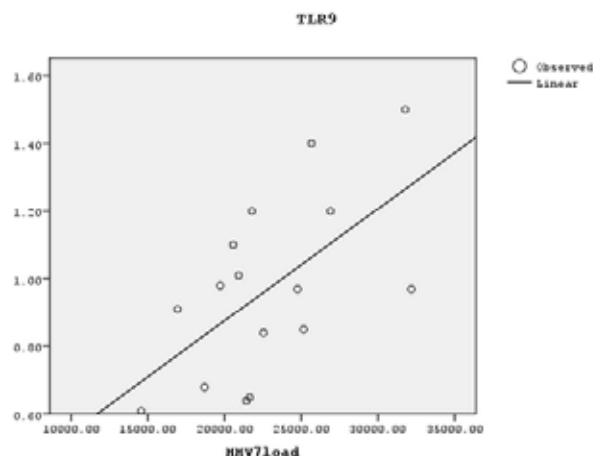


Figure 2. Positive correlation between Toll-like receptor (TLR) 9 expression and human herpesvirus (HHV) 7 viral load in pityriasis rosea cases.

Table 3. Summary of the descriptive data of Toll-like receptors within the patients group

	HHV6 positive cases (n=10)	HHV6 negative cases (n=14)	P value	HHV7 positive cases (n=16)	HHV7 negative cases (n=8)	P value
TLR2 (mean ± SD)	(1.97±0.43)	(1.97±0.55)	0.961	(2.22±0.28)	(1.47±0.44)	<0.001*
TLR3 (mean ± SD)	(9.86±1.42)	(9.92±2.02)	0.929	(9.93±1.84)	(9.83±1.69)	0.894
TLR4 (mean ± SD)	(0.84±0.33)	(1.06±0.32)	0.116	(1.16±0.22)	(0.59±0.15)	<0.001*
TLR7 (mean ± SD)	(1.48±0.49)	(1.64±0.42)	0.386	(1.57±0.48)	(1.59±0.4)	0.896
TLR8 (mean ± SD)	(5.32±1.28)	(5.01±1.35)	0.574	(5.26±1.48)	(4.88±0.89)	0.515
TLR9 (mean ± SD)	(1.02±0.39)	(0.89±0.29)	0.348	(0.97±0.26)	(0.89±0.46)	0.585

HHV: human herpesvirus; n: number; TLR: Toll-like receptors; SD: standard deviation; * $P < 0.05$ was considered significant

In this study, a significantly higher expression of TLR 2, 3, 4, 7, 8, and 9 was documented, as well as a significantly higher viral load for both HHV-6 and 7 in PR cases in comparison to the controls. Furthermore, a link between HHV presence and TLRs expression in PR was highlighted through the detection of a significantly higher expression of TLR 2 and 4 in HHV-7 positive cases in comparison to the negative cases, and also through the significant positive correlation between TLR9 expression levels and HHV-7 viral load in our cases.

The positivity of HHV-6 and/or HHV-7 in the majority of the included patients could support the viral etiology in PR. Until now, there has been no consensus on whether PR is a consequence of primary infection or due to virus reactivation. Primary HHV-6 and 7 infections mainly occur in early childhood. A primary infection in seronegative adults and in some rare cases a reinfection with the virus or reactivation from a latent state can cause the onset of PR (3,5). The presence and quantity of low and high avidity immunoglobulin G (IgG) antibodies to distinguish between primary and secondary (reinfection/reactivation) HHV infection in patients with PR could be helpful (12). Detection of virus DNA in serum can also be a marker of a productive infection (13).

Skin lesions of PR would not be due to direct infection of skin cells, but rather a reactive response to systemic viral replication (14). Both HHV-7 and HHV-6 are known to infect and reside in circulating CD4+ lymphocytes, which are the site of virus latency (15). CD4+ lymphocytes migrated to the skin could be the likely source of viral mRNA detected in our study (5). The transepidermal elimination of lymphocytes and Langerhans cells activated by viral antigens together

with increased concentrations of their chemoattractants have been suggested to be responsible for PR (16). Another suggestion supported by our results is that HHV might be involved in the pathogenesis of PR via TLR pathways.

This is in agreement with other studies (3-5,17,18) that reported a role of HHV-6 and HHV-7 in PR. In addition, several studies showed the value of antiviral treatment in patients with PR, hastening their recovery and providing further evidence for the viral etiology of PR (2).

On the other hand, other studies showed uncertain results for the positive role played by HHV-6 or 7 in PR pathogenesis (19). Such contradictory results could be attributed to the lack of sensitive measurement techniques for active viral infection (5).

Drago *et al.* (14) suggested that HHV-7 reactivation occurs during PR and that PR might be the clinical presentation of this reactivation. Our study supports preferring HHV-7 over HHV-6 in our PR cases. The present study also showed that TLR 2 and 4 expressions were higher in HHV-7 positive cases, while HHV-6 positive cases did not show a significant increase in their expression. Interestingly, a possibility of an interaction between HHV-6 and 7 has been suggested, claiming that HHV-6 can be reactivated through HHV-7 infection (20). The present results could point towards the possibility of having mainly HHV-7 coexistence involved in PR, with further activation of HHV-6 infection in some cases. As for cases with only HHV-6 positivity, the question remains whether this was a random condition or not.

The results of the current study are in agreement with others (10) that found the expression of TLR 3,

7, and 9 to be significantly elevated in the peripheral blood (PB) lymphocytes of patients with PR in comparison to normal controls, however, unlike our study, this was not evident in the skin. This discrepancy between skin and PB lymphocytes may support the concept of PR being a systemic viral exanthem with primary and early burden on the immune system.

TLR 3, 7, 8, and 9 have been shown to be involved in responses to viral infection (21), but no studies referred to their involvement with HHV. The use of TLR 3, 7, 8, and 9 analogs as antiviral therapy supports the role of TLR in viral infections (22,23). In the present study, a significant positive correlation between TLR9 expression levels and HHV-7 viral load was detected.

The possible role of TLRs in PR can be attributed to several pieces of evidence. Vercaemmen *et al.* (24) showed that TLR3 triggering activates specific signaling pathways that mount an effective immune response through the induction of cytokines and other pro-inflammatory mediators. These mediators may participate in enhancing inflammation in the affected PR area. Renn *et al.* (25) also found that after the stimulation of TLR3, and to a lesser extent TLR 7 and 8, Langerhans cells are stimulated, which were found to be increased in lesions of PR and produce large amounts of chemoattractants which may participate in the inflammation present in PR.

To the best of our knowledge, this is the first study to deal with TLR 2 and 4 in PR. Though TLR2 can recognize microbial components (26), it has been highlighted for its role in viral infections (9). Likewise, TLR4 was shown to react to viral envelope glycoprotein of certain viruses (10). It was further shown to induce gene expression involved in the inflammatory process that can point to the inflammatory reaction detected in PR (27). Furthermore, Murakami *et al.* (28) found a link between HHV-6 and TLR4; they reported significant alteration in TLR4-induced cytokines levels with HHV-6 infection. In the present study, we detected a significant elevation of both TLR 2 and 4 in HHV-7 positive patients with PR.

Importantly, the fact that TLRs are involved in the initiation of innate and acquired immune response as a response to many other pathogens precludes us from asserting that their high expression points to HHV-6 and 7 involvement in particular. Nevertheless, the detection of a significant positive correlation between TLR9 expression levels and HHV-7 viral load, as well as the significantly higher expression of TLR 2 and 4 in HHV-7 positive patients, suggest a possible link between the TLRs and HHV-7.

CONCLUSION

The present study indicated that in addition to TLR 3, 7, 8, and 9, TLR 2 and 4 also have a positive role in PR. Furthermore, HHV-6 and 7 showed a significant association with PR pathogenesis with a preference for HHV-7 over HHV-6. Future studies on the possible triggering factors for HHV reactivation in PR are strongly recommended. HHV might also be involved in the pathogenesis of PR via TLR pathways. Studying the link between HHV and TLRs in persistent cases of PR (>12 weeks) is also recommended. Closer follow-up of pregnant women developing PR during the first 15 weeks of gestation is also recommended, as PR developing during this period has been reported to be followed by unfavorable pregnancy outcomes, especially in patients presenting with atypical PR forms due to their possible association with active HHV infection (29).

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