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Detection of Human Herpes Simplex virus 6&7 in pityriasis rosea and the efficacy of acyclovir treatment.

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Abstract

Background: The exact aetiology of pityriasis rosea (PR) is still unknown as various researches exist aiming to explain the exact cause. The role of Human Herpes Virus(HHV)-6 & HHV-7 DNA in PR patients has been evaluated in many studies with variable results. The rationale behind the use of antiviral in PR is that the course of the disease follows that of a viral exanthem and also probable involvement of HHV 6 and 7 in its etiopathogenesis. The current case-control study aimed at evaluation of the possible relation between HHV-6 & HHV-7 and PR through detection of HHV-6 & HHV-7 DNA in PR patients in comparison to controls and also illumination the therapeutic efficacy of Acyclovir in PR treatment. ***Patients and methods***: This study recruited 40 subjects, 30 PR patients and 10 healthy controls. All patients were subjected to detailed history taking and examination to determine the presence of constitutional symptoms, prutitus, the type of PR and ensuring the duration of PR together with lack of drug intake in the last two weeks. Skin biopsies were taken from all subjects to detect HHV-6 & HHV-7 DNA. We initiated a comparative control study to detect HHV-6 & HHV-7 DNA in lesional and non-lesional skin samples in PR patients by nested PCR. The results of nested PCR of lesional and non-lesional skin samples were compared to well-matched healthy controls. Also, the study included another comparative control study to evaluate the efficacy of oral Acyclovir (in adult 400 mg five times a day and in children 20 mg /kg/day in five divided doses) for treatment of PR patients. The results of oral acyclovir therapy in group 1 were compared to those in a well matched group II receiving no treatment apart from emollients. ***Results***: The results revealed significant presence of HHV-6 DNA in PR lesions in comparison to controls with p=0.035, thus, support a possible relationship between HHV-6 and PR, but there was no significant difference in HHV-7 DNA in PR lesional skin compared to controls *(P=0.047)*. Also, there was significant difference between lesional HHV-7 positive and negative cases regarding the course of the disease by the end of the second week with P-value < 0.05. In addition, there was significant improvement in PR in group 1 patients with acyclovir treatment (P= 0.05) in comparison to patients in group II without acyclovir treatment (P>0.05) by the end of the second week. ***Conclusion***: HHV-6 and HHV-7 may play a role in the pathogenesis of PR and oral acyclovir is helpful in decreasing the severity and shortening the course of the disease in some patient.

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INTRODUCTION

Pityriasis rosea (PR) is an acute self-limited inflammatory disease characterized by papulosquamous skin eruption with fine scales .[1] Diagnosis of PR is generally easy to be established by clinical examination by a herald patch, typical morphology and distribution pattern of the lesions. [2.] The exact cause of the disease is still unknown. It may come from likely source of infection and marked by distinctive skin lesions with minimal constitutional symptoms.[3]Some studies incriminate Human Herpes Viruses (HHV), mainly HHV7 and HHV6, as a causative agents in patients of PR as found by positive PCR for viral DNA in the skin, plasma and peripheral blood mononuclear cells samples.[4]

On the same background, acyclovir therapy may be given in treatment of PR in light of allegations of its causing by HHV virus.[1] Rassai et al; have been tried acyclovir treatment on a group of patients with PR, they found a reduction in the erythema and the scales also resolved more rapidly.[6]

Aim of the work:

The aim of the present study was to detect a possible role of both HHV-6 and HHV-7 in the aetiology of PR patients and determination of the therapeutic efficacy of systemic use of acyclovir in cases of PR either positive or negative for both viruses.

Patients and methods:

This work was designed as prospective case controlled study.

Subjects:

Inclusion criteria of patients:

1- All patients who have history of eruption dating one week or less& diagnosed to have PR using the following diagnostic criteria of PR:[8,7]

Essential clinical features (all should be present):

1. Discrete trizonal circular or oval lesions.
2. Scaling (collarette) on most lesions.

Optional clinical features (at least one has to be present):

1. Truncal and proximal limb distribution, with less than 10% of lesions distal to mid-upper-arm and mid-thigh.
2. Orientation of most lesions along direction of the ribs.
3. A herald patch (not necessarily the largest) appearing at least two days before the generalized eruption.

2- Patients of both sexes.

3- Patients of any age.

Exclusion criteria:

1. Pregnant or lactating woman.
2. Patients who have sensitivity to acyclovir.
3. Patients with renal or hepatic impairment.
4. Patients already on antibiotics, antivirals or long- term medication (e.g. for hypertension, diabetes, hypothyroidism,.. etc).
5. Patients with co- morbid skin conditions like psoriasis, eczema or tinea corporis.

All participants in this study were subjected to the following:

1. Full history including:
   - Personal history; name, age, gender, residence and occupation.
   - Duration of the disease.
   - Association with pruritus.

- Presence of constitutional systemic symptoms including fever, headache, arthromyalgia and sore throat.
- Family history of the same or other skin diseases.
- History of drugs causing pityriasiform eruption such as: acetyl salicylic acid, barbiturates, bisthmus, clonidine, gold & omeprazole.

2. Examination; including:

- Type of PR: typical (classic) or atypical (papular, generalized, urticarial, inverse &purpuric).
- Number & Distribution of the lesions.

Thirty PR patients of age and sex matched were included and randomly divided into two groups and treated for 10 days:

a. Group I (n=20 patients) was treated with oral Acyclovir with dose of; Adults: 400mg five times a day, Children: 20mg/kg/day in five divided doses for ten days. In addition to topical emollient application.

b. Group II (n=10 patients): No treatment was given apart from emollient.

- Both groups; patients were followed up every week for 4 weeks for detection of the efficacy of treatment as disappearance of pruritus and clearance of rash.

Methods:

Samples:

From every patient with PR one punch biopsy (2mm) was taken from the lesions, also a punch biopsy was taken from non-lesional skin of only 10 randomly selected patients. 10 biopsies from healthy controls of matched age & sex (obtained from surgical procedures) were also included in the study. The lesional, as well as, the non-lesional biopsies and biopsies from controls were put in saline and stored at -70^{o}c until the time of PCR.

DNA Isolation and detection of HHV-6 and HHV-7 by nested PCR:

DNA extraction from skin biopsy samples was carried out by the use of Quick-DNA™ Universal Kit as described by manufacture instructions.

Preparation of samples:

1. 1,060 µL of storage buffer was added to each 20 mg tube of proteinase k.
2. Each tissue sample was removed from saline, and put in a new sterile eppendorf tube.
3. A solution of 95µl water,95µl solid tissue buffer (Blue), and 10µl proteinase k was added to each tissue sample.
4. All samples were incubated at 55◦c for about two hours, with grinding of the tissue every half an hour using sterile pipette tip, and returning the eppendorf back to the incubator till the end of the two hours or homogenization of the tissue.
5. After homogenization, centrifugation at 14,000 x g for 1 minute was performed to remove insoluble debris.
6. The supernatant was transferred to a new eppendorf tube.
7. 400µl Genomic Binding Buffer were added to the 200 µl supernatant and mixed thoroughly.
8. The mixtures were transferred to Zymo-Spin™ columns (in a Collection Tube),

and separately centrifuged at 14,000 x g for 1 minute, then the collection tube with the flow through was discarded.

9. Four hundred µl of DNA Pre-Wash Buffer were applied to the column in a new collection tube and centrifuged for 1 minute and the collection tube was emptied (flow through was discarded).
10. Then 700 µL g-DNA Wash Buffer were added and centrifuged for 1 minute and the collection tube emptied again.
11. 200 µl g-DNA Wash Buffer was added and centrifuged for 1 minute and the collection tube with the flow through was discarded. Finally, 50µl Elution Buffer was used to elute the DNA in a clean microcentrifuge tube (incubated for 5 minutes and centrifugation for 1 minute).

Detection of HHV-6 and HHV-7 by nested PCR:

DNA amplification by nested PCR was done using 2 rounds of amplification. In the first round of amplification, 50µl PCR reaction included:25 µl Mix,1µl of sense primer, 1µl of antisense primer, 5µl DNA template and 38 µl water. The thermal cycles were adjusted as follow: initial denaturation for 4 minutes at 95ºc, 30 cycles of denaturation at 94ºc for 30 s,annealing at 56ºc for 30 s and extension at 72 ºc for 30 s (for HHV-6). The HHV-7 DNA cycling condition involved initial denaturation at 95ºc for 4 minutes, denaturation at 95ºc for 15 s, annealing at 54ºc for 30 s and extension at 72 ºc for 30 s for 30 cycles.

After the first round of amplification, 2 µl of the first PCR product was applied to the second round PCR mixture with 1 µl of each internal primer. 30 cycles of amplification using cycling parameters was done. subsequently ,the nested PCR products of size 186-bp for HHV-6 and 264-bp for HHV-7 were confirmed on 2% agarose gel and stained with ethidium bromide in comparison to molecular size marker (φ X 174 DNA/ Hae III).

Results:

This case-controlled study was conducted on 40 subjects who were included 30 patients with PR and 10 age and sex matched normal subjects who served as controls.The patients were 15 (50%) females and 15 (50%) males, their ages ranged between 5 and 53 years with median of 21.5 years (Mean= 24.57±10.84). The normal control subjects were 5 (50%) females and 5 (50%) males. Their ages ranged from 12-42 years with median of 19.5 years (Mean= 22.9±9.73). There was no statistical significant difference in age and sex in PR patients as compared to controls with p-value > 0.05. In this study HHV-6 DNA was detected by nested PCR in 10 (33.3%) out of 30 lesional punch biopsies of PR patients and in 2 (20%) out of 10 non-lesional punch biopsies. There was a significant statistical difference in HHV-6 lesional PCR results in patients group compared to control group with p= 0.035. HHV-7 DNA was detected by nested PCR in 6(20%) out of 30 lesional skin biopsies and all the non- lesional skin biopsies were negative. In control group, all the samples were negative for HHV-6& HHV-7 (Table 1).

Table (1): Distribution of subjects according to PCR results.

	Cases		Control		Test of significance
PCR lesional	**N=30**		**N=10**		
	N	%	N	%	
HHV6	10	33.3	0	0.0	x^2=4.44 p=0.035*
HHV7	6	20.0	0	0.0	FE p=0.47
PCR non lesional	**N=10**		**N=10**		
	N	%			
• **HHV6**	2	20.0	0	0.0	FE p=0.47
• **HHV7**	0	0.0	0	0.0	…….

$X^2$for Chi-square test
FE for Fischer Exact test
* P value significant ≤0.05*

Thirteen ~~13~~ patients (65%) with lesional HHV-6 negative results were included in group I (on acyclovir treatment) and 7 patients (35%) were included in group II (No acyclovir treatment). 7 patients (70%) with lesional HHV-6 positive results were included in group I and 3patients (30%) were included in group II. There was no significant difference between patients with HHV6 lesional negative and positive as regards treatment received with P-value > 0.05(table 2).

Table (2): Comparison between cases with lesional HHV6 positive and negative regarding age,sex, clinical presentation and treatment received.

Parameters	HHV6 Lesional		Test of significance	p value
	negative n=20 N(%)	Positive n=10 N(%)		
Age (years)				
Median (min-max)	21.5(5-50)	21.5(14-53)	Z=0.164	0.87
Sex				
Male	12(60)	3(30)	χ^2=2.4	0.12
Female	8(40)	7(70)		
constitional symptoms	5(25)	3(30)	χ^2=0.08	0.77
Pruritus	12(60)	10(100)	χ^2=3.97	0.019*
Type of PR				
Papular	1(5)	1(10)	Monte Carlo test	0.51
Classic	15(75)	8(80)		
Inverse	2(10)	0(0)		
Urticarial	0(0)	1(10)		
Generaized	1(5)	0(0)		
Purpuric	1(5)	0(0)		
Duration of PR(days) (mean±SD)	6.27±1.67	6±1.85	t=0.39	0.7
Treatment received				
• **Group I**	13(65)	7(70)	χ^2=0.075	0.784
• **Group II**	7(35)	3(30)		

Z for Mann Whitney U test X^2 for Chi square test FE for Fischer Exact test
* P value significant ≤0.05*

Seven patients (87.5%) with non lesional HHV-6 negative results were included in group I and 1 patients (12.5%) were included in group II. The 2 patients (100%) with non lesional HHV-6 positive results were included in group II. There was no significant difference between patients with HHV6 non-lesional negative and positive as regards treatment received with P-value > 0.05(table 3**).**

Table (3): comparison between cases with non lesional HHV6 positive and negative regarding age, sex, clinical presentation and treatment received.

Parameters	HHV6 non-lesional		test of significance	p value
	Negative n=8 , N(%)	Positive n=2, N(%)		
Age				
Median(min-max)	20.5(17-28)	34(18-50)	Z=0.53	0.59
Sex				
• **Male**	7(87.5)	2(100)	FE	1
• **Female**	1(12.5)	0		
constitional symptoms	2(25)	0	FE	1
Pruritus	6(75)	2(100)	FE	1
type of PR				
• **classic**	7(87.5)	1(50)	FE	0.38
• **inverse**	1(12.5)	0	FE	1
• **urticarial**	0	1(50)	FE	0.2
Duration of PR(days)	7(2-7)	7(7-7)	Z=0.75	0.46
treatment received				
• **Group I**	7(87.5)	0	FE	0.07
• **Group II**	1(12.5)	2(100)		

Z for Mann Whitney U test FE for Fischer Exact test

Sixteen patients (66.7%) with lesional HHV-7 negative results were included in group I and 8 patients (33.3%) were included in group II. Four patients (66.7%) with lesional HHV-7 positive results were included in group I and two patients (33.3%) was included in group II. There was no significant difference between patients with HHV7 lesional negative and positive as regards treatment received with P-value > 0.05(table 4).

Table (4): comparison between cases with lesional HHV7 positive and negative regarding age,sex, clinical presentation and treatment received.

Parameters	HHV7 Lesional		test of significance	p value
	Negative n=24, N (%)	positive n=6, N (%)		
Age (Years)				
median(min-max)	22.5(12-53)	20.5(5-29)	Z=0.83	0.41
Sex				
Male	11(45.8)	4(66.7)	FE	0.65
Female	13(54.2)	2(33.3)		
Constituional symptoms	7(29.2)	1(16.7)	FE	1
Pruritus	17(70.8)	5(83.3)	FE	1
Type of PR	n=24	n=6		
Papular	2(8.3)	0(0)	Monte Carlo test	0.162
Classic	19(79.2)	4(66.7)		
Inverse	2(8.3)	0(0)		
Urticarial	0(0)	1(16.7)		
generalized	0(0)	1(16.7)		
Purpuric	1(4.2)	0(0)		
Duration of PR(days) mean±SD	6±1.84	7±0	t=1.31	0.2
treatment received				
• **Group I**	16(66.7)	4(66.7)	FE	1
• **Group II**	8(33.3)	2(33.3)		

t for independent t test FE for Fischer exact test Z for Mann Whitney U test

According to PCR results, 8(40%) patients were positive for HHV-6 in lesional skin samples in group I, 4(20%) patients were positive for HHV-7 in lesional skin samples and no positive results for both viruses in non lesional skin samples. Regarding group II, 2(20%) patients were positive for HHV-6 in lesional skin samples, 2(20%) patients were positive for HHV-7 in lesional skin samples, 2(20%) patients were positive for HHV-6 in non- lesional skin samples and no positive results for HHV-7 in non lesional skin samples (table 8). There was no significant difference between the 2 groups in age &sex distribution, pruritus, type of PR, duration of PR & PCR results with P-value >0.05 but, there was significant difference between the 2 groups in constitutional symptoms with P-value < 0.05 (table 5).

Table (5): comparison between treatment groups regarding age, sex, clinical presentation and PCR results.

parameters	Treatment group		test of significance	p value
	Group I **n=20**	Group II **n=10**		
	N (%)	**N (%)**		
Age (years)				
Median (min-max)	21.5(5-53)	20(12-50)	Z=0.31	0.76
Sex				
Male	9(45)	6(60)	χ^2=0.6	0.44
Female	11(55)	4(40)		
constitional symptoms	8(40)	0	χ^2=5.46	0.02*
pruritus	16(80)	6(60)	χ^2=1.36	0.24
type of PR				
• **Papular**	2(10)	0(0)	MC	0.0102
• **Classic**	16(80)	7(70)		
• **Inverse**	0(0)	2(20)		
• **Urticarial**	0(0)	1(10)		
• **Generalized**	1(5)	0(0)		
• **Purpuric**	1(5)	0(0)		
Duration of PR(days) Median (min-max)	7(2-7)	7(2-7)	Z=1	0.31
PCR results				
HHV6 lesional positive	8(40)	2(20)	χ^2=1.2	0.27
HHV7 lesional positive	4(20)	2(20)	no p value because constant	……
HHV6 non- lesional positive	0(0)	2(20)	FE	0.07
HHV7 non- lesional positive	0(0)	0(0)	no p value because constant	…….

*P value significant ≤0.05 χ^2 for Chi square test FE for Fischer Exact test Z for Mann Whitney U test

Discussion:

PR is a papulosquamous disease characterized, in its typical form, by an initial lesion, herald patch, followed after 1-2 weeks by a generalized secondary rash. Although PR is a self-limited disease and lasts for 6-8 weeks, the associated pruritus and the appearance of the rash cause much distress to the patients [1]. The exact pathogenesis is unknown, but the most accepted theory suggests a systemic immune inflammatory response in a genetically predisposed person which is triggered, mostly, by a viral agent [9]. Many epidemiological and clinical features suggest a

viral pathogenesis. They include preferential occurrence of PR in spring, fever as prodromal symptom, familial clustering in some cases and presence of herald patch (which may be correlated with the inoculation point of organism) followed by secondary eruption, characteristic distribution, spontaneous regression of skin lesions and recurrences during immunosuppression, as is often observed in patients with viral diseases.[10,11]

The aim of this study was to evaluate the possible role of HHV-6&HHV-7 as aetiology in PR patients, in comparison to age and sex-matched healthy individuals and the effect and the therapeutic role of oral -acyclovir in patients with PR either positive or negative for both viruses.

The present study comprised 30 patients with PR and 10 age and sex matched controls. The patients group included 15 (15%) males and 15 (50%) females, PR patients were likely to be females and males equally that agrees with ***Engin et al.***[12] who reported that both sexes are equally affected. These findings were in contrast to ***Kirac et al.***[13] who found that PR is more prevalent in females up to 1.5 times than males. Ages of patients ranged from 5 years to 53years with median of 21.5 years. One (3.3%) patient was less than 10 years of age, 24 (80%) patients were between 10 to 35 years of age and 5 (16.7%) patients were more than 35years of age. This age distribution supports the view of ***Burns et al.***[14] who stated that most cases of PR occur between the ages of 10 and 35 years and it is uncommon in infancy, early childhood or old age. In our work, all patients were included if they have their onset of the disease only within 7 days or less before samples taking and before the start of Acyclovir therapy, while in ***Taher and Moustafa,***[15] study, samples were taken on an average of 10.5 days from the disease onset and in ***Amatya et al.***[16], the duration of the disease before starting Acyclovir therapy varied between 7 -14 days. The presentation within 7 days of the disease onset seems a reasonable criterion for patients' selection in early stages, as was done in our work, so that by the end of Acyclovir treatment we are still before the expected natural resolution of PR rash.

There was no significant difference between HHV-7 in lesional skin biopsies and healthy control, with P-value > 0.05. This is in agreement with ***Kempf et al.***[17] who had detected HHV-7 in only 1 out of 13 (8%) skin biopsies of PR lesions versus 2 out of 14 (14%) of normal skin of control. They concluded that low detection rate of HHV-7argues strongly against a causative role of HHV-7 in the pathogenesis of PR. ***Offidani et al.***[18] investigated the presence of HHV-7 in scales of lesions from 12 patients with active PR in parallel with 20 healthy controls. They could not detect HHV-7 DNA sequences in scales of lesional skin of PR as well as in skin of control. These findings could not support the idea of a correlation between HHV-7 and PR.

In a study done by ***Akar et al.*** [19], HHV-6 DNA sequences were detected in 8 out of 32 (25%) skin samples of patients with PR. Our results for HHV6 in lesional skin of PR patients; 10/30 (33.3%) skin samples agreed with these findings. On contrary, our results for HHV-7 disagreed with that of Akar et al.[19] who did not detect HHV-7 DNA in skin lesions of any patient with PR. They concluded that HHV-6 DNA positivity in primary and secondary lesions may suggest that the HHV-6 may play a part in pathogenesis of some patients with PR.

Because of the disparate findings in the literature and the high degree of genetic similarity between HHV-7 and HHV-6, we were extremely cautious in our selection of virus specific primer sets. Our results provide firm evidence that both HHV-7 and HHV-6 were reactivated in patients with PR. This evidence supported a study done by ***Canoplat et al.***[20] who have found HHV-6 and HHV-7 DNA sequences in skin samples of PR patients. The number and percentage of positive cases in our study were near to that study. In this study, encouraging results were obtained using acyclovir. By the end of the fourth week, 13patients (65%) of group I had complete response as opposed to 4 patients (40%) of group II. This implies that acyclovir reduce the course of the disease. This is in agreement with ***Eshani et al.***[21] ***& Amatya et al.***[16] who reported that after eight weeks and after 4 weeks respectively, the rate of complete response in patients who received high-dose acyclovir was significantly higher than their matches who received oral erythromycin.

In the present study, 30% (6 out of 20) patients treated with oral Acyclovir had complete response by the end of the second week of therapy. There was significant difference between group I and group II regarding the course of the disease by the end of the second week with p =0.05. This is in agreement with ***Amatya et al.***[16] who reported that 38.8% (7 out of 18) treated with oral acyclovir had complete response by the end of second week of therapy. However, a placebo control trial done by ***Drago et al.***[22] who found that 79% of patients treated with oral acyclovir had complete response compared with 4% of the placebo group by the end of the second week of therapy.

Conclusion:

Based on the number of positive cases for HHV-6 and HHV-7 in the results of the present study on 30 PR patients, we can conclude that HHV-6 and HHV-7 may play a part in the pathogenesis of PR in some patients. Although PR is a self-limited disease which resolves within three weeks to three months, this study revealed that the difference between the treatment groups regarding the course of the disease was statistically significant and oral acyclovir is helpful in rapid decreasing the severity and shortening the course of the disease in some patients, making those patients satisfied with the rapid cure. This clinical trial, although small, suggest a therapeutic benefit from oral acyclovir in shortening the course of the disease**.**

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