Prevalence of Human Papilloma Virus Type 5 DNA in Psoriatic Patients: Association with Disease Severity and Exposure to Phototherapy

Sahar Taher1, Sanaa Mohie Eldin1, Magie Mosbah1 and Abeer M. AbdelAziz2

Departments of Medical Microbiology and Immunology1, Dermatology, Venerology and Andrology2, Faculty of medicine, Mansoura university, Egypt

INTRODUCTION

Human papillomaviruses (HPVs) are small epitheliotropic DNA viruses that can induce cutaneous and mucosal lesions and appear to be closely linked to skin cancers. A broad variety of HPV types which have been referred to as epidermodysplasia verruciformis (EV) HPV's. It was found that patients with psoriasis revealed high prevalence of EV-associated HPV, suggesting that EV-HPV can act as putative antigen contributing to the pathogenesis of psoriasis. In particular, some viral genotypes HPV-5 and HPV-36 have been associated with psoriasis. Objective: To assess the presence of HPV-5 DNA in skin of psoriatic patients, its relation to disease severity by using PASI score and correlation with phototherapy treatment. Design: Screening for the presence of HPV-5 DNA sequences in skin biopsies and scrapings from psoriatic patients without treatment (group A), with history of Psoralen-ultraviolet A (PUVA) treatment (group B), with narrow band ultraviolet B (NB-UVB) (group C) and skin biopsies from control (group D). Patients and methods: Samples were taken from 54 patients with plaque type psoriasis (39 men and 15 women, mean age 52.8 years) including 25 patients in group A, 15 patients in group B (mean number of PUVA exposure 140), 14 patients in group C (mean number of NB UVB exposure 60) and 15 subjects in group D. DNA was isolated from skin samples and analyzed by polymerase chain reaction with the use of 2 nested primer systems specific for HPV type 5. Results: The rate of HPV DNA positivity was significantly higher in patient groups than control group with a percentage of 24% (6/25) in group A, 33.33% (5/15) in group B, 28.5% (4/14) in group C and 6.6% (1/15) in group D (P=0.003 and X²=25.9). There was a significant difference between lesional and non-lesional areas of psoriatic patients regarding HPV-DNA positivity with P < 0.0001 and X²= 16.98. PASI score was highly significant P < 0.001 between HPV-5 DNA negative, positive and double positive psoriatic patients. Conclusion: Skin of psoriatic patients was a reservoir for HPV-5. The prevalence was high in lesional than non-lesional areas, while HPV-5 negative subjects seemed to have a less severe disease. PUVA and NB UVB treatment for psoriasis in optimum doses are not associated with increasing HPV-5 in psoriatic patients.
methotrexate, topical tar and arsenic (12). A third theory is that PUVA may promote tumorigenic viral agents such as HPV (13).

Some findings have revealed that HPV DNA especially HPV type 5 was found in involved skin of patients with psoriasis and in skin of patients treated with PUVA phototherapy, thus, suggesting that EV HPV can act as putative antigen contributing to pathogenesis of psoriasis (14 and 15). Others, however, have shown that EV HPVs are widespread and detected at high prevalence in normal skin of general population, thus suggesting a commensalic nature of these viruses (16, 17 and 18).

In the present work, we aimed to assess the presence of HPV-5 DNA in psoriatic patients and its relation to disease severity and correlation with PUVA and NB UVB treatment.

**MATERIALS AND METHODS**

**Patients and control:**

Fifty four patients (39 males and 15 females) with plaque-type psoriasis were recruited from patients seeking treatment for psoriasis at the Dermatology Department and from outpatient clinic of Dermatology at Mansoura University hospitals. Their age ranged from 18-78 years (mean 52.8±4.3). The patients were classified into 3 groups: group A, 25 non-treated patients (18 males and 7 females), group B, 15 patients (12 males and 3 females) with history of PUVA treatment, the mean number of PUVA exposure was 140 (range 17-285), the mean total UVA dose was 464 J/cm² (range 44-750), group C, 14 patients (9 males and 5 females) with history of NB UVB treatment, the mean number of NB UVB exposure was 60 (range 4-83). We excluded patients with present, past or family history of warts, autoimmune diseases and benign or malignant tumors.

**Table (1): Gender and age of the studied patients and control**

<table>
<thead>
<tr>
<th>Gender (m/f)</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>18/7</td>
<td>12/3</td>
<td>9/5</td>
<td>10/5</td>
</tr>
<tr>
<td>Age Range</td>
<td>50.2±8.5</td>
<td>50.1±3.9</td>
<td>53.1±4.21</td>
<td>53.19±6.1</td>
</tr>
</tbody>
</table>

Chi-square test between all gender groups \( \chi^2 = 3.1 \) \( P = 0.51 \)

One way Anova F test \( F = 2.09 \) \( P = 0.25 \)

The psoriatic area and severity index (PASI) score were calculated (19) for each patients and varied between 5.6 to 70 (mean 25.9±12). Diagnoses were made on the basis of patient history and clinical inspection. Fifteen subjects with healthy skin, matched age and gender with the patient groups and served as control group (group D).

**Samples:**

From each patient and control, a 5mm punch biopsy was taken at site mostly affected by psoriasis (lesional skin) after obtaining their consent, another biopsy was taken from non-lesional skin. Scraping from scales in the lesional skin were taken using sterilized scalpel. Skin areas from which tissue biopsy and scales obtained were similar (neck, forearm, arm, thigh, buttock, trunk, hand, wrist, elbow). All samples, biopsies and skin scraping were put in ependorf containing phosphate buffered saline and kept in deep freezing -70°C until DNA extraction and amplification were done.

**DNA preparation and detection of HPV-5 DNA**

by nested PCR:

DNA preparation:

By the use of QIA Amp Kit (Qiagen Hilden, Germany)

DNA extraction:

The use of QIA Amp Kit (Qiagen Hilden, Germany)

Twenty \( \mu L \) Qiagen protease were pipetted into the bottom of a microcentrifuge tube.

Two hundred \( \mu L \) of samples after grinding with tissue grinder in phosphate buffer saline (PBS) and buffer AL (200 \( \mu L \)), were added and mixed by pulse vortexing for 15 seconds. The mixture was incubated at 56°C for 10 minutes, then 200 \( \mu L \) ethanol were added and mixed. The mixture was applied to the spin column and centrifuged at 8000 rpm for 1 minute. Five hundred \( \mu L \) of AW2 were applied and centrifuged at 14000 rpm for 3 minutes was done. Spin column was placed in microcentrifuge tube and collection tube containing the filterate was discarded. Two hundred buffer AE was added, incubation at room temperature and centrifugation were done. A second step with further 200 \( \mu L \) AE buffer will increase yields by up to 15%

DNA amplification by nested PCR (Prignano et al. (15) for amplification of HPV-5 by nested PCR, 2 rounds of amplification were done. In the first round of amplification, \( 1 \mu L \) of each nucleotide primers 5'-GGAAGCCTGCGAATTC-3' (position 360 – 381 of HPV genome) and 5'-CTTCCAGGCCTCCTCACCT-3' (position 620 – 639 of HPV) generates a 280-bp product, were used. Two \( \mu L \) of each DNA preparation, 1 \( \mu L \) of sense primer, 1 \( \mu L \) of
anticense primer were added to 46 µL of master mix containing 3 µL MgCl2 solution, 5 µL X PCR buffer (100mM Tris HCl (pH 8.3), 500 mM KCl/µL), 1 µL d NTPs mixture (10mM/µL), 0.4 µL Ampli Taq DNA Polymerase (5 units/ml) all in 36.6 µL distilled water. The reaction was overlaid with mineral oil. After an initial denaturation step of 5 minutes at 94°C, 38 cycles were performed at 94°C for 45 seconds, 54°C for 45 seconds and 72°C for 1 minute.

After the first round of amplification, 2 µL of the first PCR product was added to the second round PCR mixture with 1 µL of each internal primers 5’- TGTGTTCGCCGTGCTGT CGC-3’ (position 414-432) and 5’- CCTCTGC CACAGCAATCTAATT -3’ (position 582 – 605) generates a 192-bp product. Twenty five cycles of amplification using the cycling parameters were performed. Subsequently, the nested PCR products of size 192-bp were confirmed on 2% agarose gel and stained with ethidium bromide in comparison to molecular size marker (φ X174 DNA/ Hae III).

Statistical analysis:
Statistical analysis was done by using SPSS (statistical package for social science program version 10, 1999). The qualitative data were presented in the form of number and percentage. Chi-square test with Yates correction was used to study difference of qualitative data, Yates correction was used for small number in some cell. The quantitative data were expressed in the form of mean, standard deviation and range. One way Anova test was used for comparison between quantitative data of the four groups. Bonferroni test was used to compare between each 2 groups for quantitative date. Significance * was considered at P value less than 0.05. Highly significance ** was considered when P value is less than 0.01. Extremely significance *** was considered when P value is less than 0.001. Insignificance was considered when P value is more than 0.05

RESULTS
Out of 54 psoriatic patients and 15 controls, the prevalence of HPV-5 DNA by nested PCR were in skin biopsies of group A 24% (6 out of 25), group B 33.33% (5 out of 15), group C 28.6% and group D 6.7%, while skin scrapping demonstrates a lower percentage in patients group (no scrapping was taking from control group).

Table (2): The percentage of HPV-5 DNA in the skin biopsy and scraping from patients with psoriasis and control

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>%</th>
<th>Group</th>
<th>No</th>
<th>%</th>
<th>Group</th>
<th>No</th>
<th>%</th>
<th>Group</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>6</td>
<td>24%</td>
<td>Group B</td>
<td>5</td>
<td>33.33%</td>
<td>Group C</td>
<td>4</td>
<td>28.5%</td>
<td>Group D</td>
<td>1</td>
<td>6.6%</td>
</tr>
<tr>
<td>Skin scrapping</td>
<td>5</td>
<td>20%</td>
<td>4</td>
<td>26.67%</td>
<td>2</td>
<td>14.2%</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>66.67%</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>19</td>
<td>76%</td>
<td>10</td>
<td>66.67%</td>
<td>10</td>
<td>71.5%</td>
<td>14</td>
<td>93.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi-square test between all groups
Χ² = 25.9 P = 0.003**

Chi-square test between each two groups

A vs D  Χ² = 9.19  P = 0.021*
B vs D  Χ² = 10.82  P = 0.015*
C vs D  Χ² = 9.93  P = 0.02*

** highly significant
*significant

Biopsies were taken from lesional and non-lesional areas from 36 psoriatic patients from different body sites including upper limbs, lower limbs, neck and trunk, table (3) illustrates this result

Table (3): Comparison between lesional and non-lesional areas of psoriatic patients in relation to different body sites.

<table>
<thead>
<tr>
<th>Body site</th>
<th>Lesional area</th>
<th>Non-lesional area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>positive</td>
</tr>
<tr>
<td>Upper limbs</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Lower limbs</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>Trunk and Neck</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>15</td>
</tr>
</tbody>
</table>

Test of significance between lesional and non-lesional areas Chi-square with Yates correction
Χ² = 16.98  P < 0.0001 ***
Table (4): Association between PASI score and HPV-5 DNA in lesional and non-lesional areas

<table>
<thead>
<tr>
<th>HPV-5 DNA</th>
<th>No. of patients (54)</th>
<th>PASI score</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>39</td>
<td>10.2±3.09</td>
<td>(5.6 – 16)</td>
<td></td>
</tr>
<tr>
<td>Positive (lesion)</td>
<td>15</td>
<td>31.7±7.81</td>
<td>(17.6 – 52)</td>
<td></td>
</tr>
<tr>
<td>Double positive</td>
<td>7</td>
<td>48.21±9.22</td>
<td>(27.8 – 70)</td>
<td></td>
</tr>
</tbody>
</table>

Test of significance between all negative, positive and double positive
One way Anova F test F = 25.1 P < 0.001***

Then Bonferroni test between each 2 groups
Negative vs Positive P = 0.002**
Negative vs Double positive P < 0.001***
Positive vs Double positive P = 0.029*

*** extremely significant
** highly significant
* significant

Figure (1): Examples of HPV-5 nested PCR products following electrophoresis in ethidium bromide-stained agarose 2% w/v gels. Lanes: M, molecular size markers, lanes 1,2,3,4 and 6 positive results at 192-bp product

DISCUSSION

Previous studies have shown that HPV-5 DNA is found frequently in individuals affected with epidermodysplasia verruciformis (EV), skin carcinogenesis, bullous diseases, some autoimmune diseases and particularly psoriasis (14). Less frequently, other HPV types have been reported, sometimes also associated with psoriatic patients (20).

It is well recognized that infections with cutaneous EV-associated HPVs are widespread in humans and that body hair follicles may represent a silent reservoir for these viruses (21). Premalignant skin lesions, cutaneous squamous cell carcinomas (SCCs), psoriatic skin scrapings and biopsy specimens have all shown a particularly high prevalence of EV HPV DNA, and this points to the possible involvement of HPV in the etiology of psoriasis as well tumorigenesis (9). Other investigators reported that anti-HPV-5 antibodies may arise in normal subjects during epidermal repair processes following burns, thus suggesting that this condition like the psoriatic lesions, is dependent upon extensive keratinocyte proliferation (20).

The present study comprised 54 psoriatic patients and 15 healthy subjects as control. They were categorized into 4 groups, group A, 25 psoriatic patients with no treatment, group B, 15 patients with history of PUVA treatment, group C, 14 patients with history of NB UVB therapy, and group D, 15 control subjects. Human papillomavirus type-5 DNA was assessed by the use of nested PCR to amplify 192-bp segments. It was found that HPV-5 DNA detected in biopsies taken from group A in 6 patients (24%), 5 patients (33.33%) in group B, 14 patients in group C (28.6%), and 1 control in group D (6.7%), while skin scraping revealed lower prevalence, 5 patients (20%) in group A, 4 patients (26.7%) in group B, 2 patient (14.3%) in group C.

In this study, the results of PCR-based screening demonstrated that HPV infection is present in skin of psoriatic and healthy individuals but with significant difference, and this in agreement with other authors (18) that EV-HPV
genotypes are widespread and found at a high detection rate among general population. On the other hand, it was found that significant higher detection rate of HPV-5 in psoriatic patients than normal control (15).

Also, higher rate of HPV-5 DNA sequences, and antibodies against HPV-5 L1 capsid protein, have been reported in psoriatic patients compared to patients with atopic dermatitis, renal transport recipients and subjects from general population (14). Moreover, in other studies, HPV-5 was the most prevalent HPV-genotype found in the skin of normal individuals worldwide (18), and was commonly detected in hair follicles of immunosuppressed and immunocompetent subjects with and without skin cancer (16).

On the contrary, Wolf et al., (22) found HPV-5 to be rarely detected in psoriatic patients and control, also Viviano et al., (23) don’t confirm the high prevalence of the genotype HPV-5 in psoriatic patients, since only 7.4% of them were found HPV-5 positive.

The reasons for these differences remain unclear, but they may involve the geographic origin of patients, type of sampling (plucked hair vs. scraped scales or biopsy) and this was obvious in our study, when we used two methods of sampling, we found that the frequency of HPV-5 detection in the biopsy was higher than in the scales. Other reason for this difference may be differences in the molecular detection techniques used.

In our study, there’s a significantly higher detection rate in psoriatic lesion than non-lesional areas in the same patient. In a study conducted by Prigano et al., (15), directed towards psoriatic skin, and the significantly higher colonization frequencies observed in the lesional areas suggested that HPV is more prevalent where inflammatory mechanisms are activated and keratinocytes are proliferating. This observation is in agreement with the finding in normal individuals that HPV-5 DNA and antibody detection rates increase concomitantly with skin repair phenomena.

Previous studies of psoriasis have also shown that even uninvolved skin undergoes various biochemical modifications, such as activation of inflammatory process (24), and this perhaps was reflected in the degree of HPV-5 DNA positivity recorded in the study for non-lesional skin. This may also indicate that the virus DNA is present in low amounts and becomes detectable only after cell proliferation has begun (20), possibly depending on pro-inflammatory cytokine release (25).

In the present work, we analyzed the HPV-5 DNA positivity in relation to skin site distribution in psoriatic patients, no statistical difference was found between different body site affection. This is in agreement with (Prigano et al., 2005) who analyzed samples taken from different skin areas didn’t show significant differences in HPV-5 DNA positivity, suggesting that the presence of HPV-5 DNA depends more on keratinocyte proliferation than on body localization. Since HPV replication is known to be activated when keratinocyte replication is active, it can be speculated that the psoriatic keratinocyte is a particularly suitable host for HPV-5 viruses which perhaps utilize a receptor molecule that is exposed particularly on psoriatic keratinocytes (15).

The PASI score was evaluated in psoriatic subjects divided into three groups according to presence of HPV-5 DNA: (1) negative, (2) positive only in lesional samples and (3) positive in both lesional and non-lesional skin samples. There was a significant difference between the groups, suggesting that the disease severity and the presence of HPV-5 were associated. Our results match that of Prigano et al. (15) who found that HPV-5 negative subjects seemed to have less severe disease.

Wolf et al. (12) found that the prevalence of HPV in plucked body hairs was significantly higher in PUVA-treated patients with psoriasis than non-PUVA treated patients (80% vs 42%) and interpreted this observation in terms of PUVA-induced immunosuppression (26) which like UV exposure, may favour the survival of HPV-infected keratinocytes or in terms of a direct stimulating influence of PUVA on HPV viral activities, UV light has recently shown to differentially regulate the promoters of a number of cutaneous HPV’s (27). On the other hand, Favre et al. (28) found no significance in HPV-5 detection rates between PUVA-treated and untreated patients.

UVR and HPV might also target related or complementary cellular and DNA repair mechanisms within the keratinocyte. For example, the early HPV proteins E6 and E7 of high-risk HPV are known to bind and inactivate the tumor suppressor gene products P53 and Rb, respectively (29). Low-risk HPV, more frequently found in skin lesions, can also bind P53 and Rb but with lower affinity than high-risk HPV and without disruption cell cycle control (30). Specific mutations within the P53 gene are produced by selected genotoxic injury, and have been designated “signature” mutations. HPV may enhance UVR genotoxic effects and mutations by disruption of cell cycle control mechanisms required for high fidelity DNA repair. Moreover, E6 protein from a range of cutaneous HPV types significantly inhibits apoptosis in response to UV damage (31).
In our study, we didn’t find a significant difference regarding detection of HPV-5 in non-treated patients versus PUVA or NB UVB treated patients. This can be explained by the lower exposure times and lower doses than that described in the study of Harwood et al. (6) and Wolf et al. (22) whose patients used prolonged PUVA therapy and thus, a high prevalence of HPV-5 was present in those patients than non-treated patients. Another reason for the difference between our and their result is that Wolf et al. (22) found higher prevalence of HPV-8 not HPV-5 and they suggested a specially high responsiveness of HPV-8 to PUVA treatment.

In conclusion, this study confirmed that skin of psoriatic patient is a reservoir for HPV-5. The prevalence was high in lesional areas than non-lesional areas, while HPV-5 negative subjects seemed to have a less severe disease. HPV may be a simple reflection of keratinocyte proliferation, the possibility that HPV-5 acts as a trigger for clinical manifestation of the disease by inducing a local immune response including proinflammatory mediators, leading to subsequent keratinocyte proliferation is an interesting possibility. PUVA and NB UVB treatment for psoriasis in optimum doses decrease keratinocyte proliferation and are not associated with increasing HPV-5 in psoriatic patients. Further studies are needed to detect the presence of HPV-5 in other dermatoses with damaged skin or hyper-proliferating keratinocytes.

REFERENCES

BY: The Egyptian Journal of Medical Microbiology, July 2007 Vol. 16, No. 3

...