Role of Human Papilloma Virus (HPV) in Common and Genital Warts and its Relation to P53 Expression

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ABSTRACT

Background and Aim: Human papilloma viruses (HPVs) are small DNA tumor viruses that infect epithelial tissues and cause warts. One of the viral genes responsible for HPV's oncogenic activity is E6 which is known to inactivate the cellular p53 tumor suppressor gene. We aim to detect the presence of HPV infection and its different types in human warts, and to identify the relation between HPV and p53 expression in skin and genital lesions.

Patients and Methods: We studied markers of HPV infection in overall of 30 patients (20 with common warts, and 10 with genital warts). Also, 30 normal skin samples were taken from each patient as a normal control. Detection of HPV was done using polymerase chain reaction (PCR), and HPV typing was performed using LiPA (Line immuno Probe Assay). In addition, all skin lesions were examined by immunohistochemistry for p53 expression.

Results: In patients with common warts, HPV DNA was found in 4/20 (20%) of cases which was of HPV types 11, 31, 6, 33 (p=0.28). Also, P53 expression was found in 4/20 (20%) of cases (p=0.26). No single patient showed reactivity of both HPV and p53 expression. In patients with genital warts, however, HPV DNA was found in 6/10 (60%) of cases. Of these, 5 cases were positive for HPV type 6 and one case had HPV type 11. Three patients (30%) were positive for p53. In the normal skin control, 2/30 (6.6%) were positive for HPV DNA which were of types 5, and 31.

Conclusions: We conclude that; (1) Prevalence rate of HPV infection in warts is higher than those of normal control group, and Egyptian patients with genital warts had higher prevalence rate of HPV than those with common warts, (2) In Egypt, HPV types 6, and 11 are the most prevalent genotypes associated with genital warts and HPV types 6, 11, 31, and 33 are associated with common warts, (3) There was no definite relation between p53 expression and HPV detection, (4) Also, there was no association between the different HPV types and p53 detection in these non-cancerous lesions.

Key Words: HPV - Warts - P53.

INTRODUCTION

Human papilloma virus (HPV) is a member of the papovaviridae family which comprises a large number of double stranded DNA viruses of approximately 8kb that are packaged into non enveloped particles of about 55nm [1]. HPV is involved in the development of a wide range of diseases from common warts to the rare epidermodysplasia verruciformis (EV) [2]. It characteristically infects epithelial cells through small abrasions caused by various forms of physical trauma. HPV infects the basal cells of the epithelium and multiply in its upper more differentiated layers of the epithelium [3]. Hyperplasia and hyperkeratosis are the hallmarks of skin infection by dermatotropic HPV. The characteristics of the lesions depend on the type of HPV causing the infection. Type 1, 2, and 4 are associated with common warts and plantar warts. Types 3, 10, 28, and 41 are associated with flat warts. Whereas, other HPV types are found in patients with EV which are types 5, 8, 9, 12, 14, 15, 17, 19-25, 36, 46, and 47 [4]. Genital infections caused by HPV, which is also
known as venereal warts or condyloma acuminate (CA) are most commonly caused by types 6 and 11 [5]. Progression to carcinoma is rare, and carcinoma of this region is associated mainly with types 16 and 18 [6].

Human papilloma virus genome has two coding regions and a non-coding regulatory region. The two coding regions are histologically divided into two groups, early (E) and late (L) genes that are clustered in separate regions. The early genes include E1, E2, E4, E5, E6, and E7, which code for proteins involved in viral DNA replication, transcriptional control, and cellular transformation. The late genes encode (L1) and (L2) are the structural proteins of the virus [6,7]. The function of E6 and E7 is to establish and maintain a cellular milieu that allows for viral replication. The E6 and E7 proteins of the high-risk HPV types, such as HPV 16 and 18, act as viral oncoproteins, but no such functions are associated with the corresponding proteins from the low-risk types such as HPV 6 and 11. In high-risk HPV types, E6 binds the p53 tumor suppressor protein as part of a trimeric complex with the cellular ubiquitin ligase, E6AP, leading to the rapid turnover of p53 and reduction of its half life from several hours to less than 20 minutes [8,9]. E7 binds to the retinoblastoma (Rb) family of tumor suppressors, as well as other proteins involved in cell cycle regulation [10,11]. In case of malignant cells, the E1/E2 ratio is changed when the virus is integrated into the chromosome of the host cell and the expression of E6 and E7 is lost. Uncontrolled expression of E6 and E7 proteins, as a consequence of viral integration, is paramount to the establishment and maintenance of the tumorigenic state. In addition, expression of E6 & E7 increases genomic instability of the host cells thus accelerating malignant progression [1].

Few studies have shown HPV infection in Egyptian patients with genital warts, but the role of HPV and its types in common and genital wart is less clear and has not been well studied. So, we aim to; (1) Study the prevalence rate of HPV infection in Egyptian patients suffering from both common and genital warts, (2) Identify the most prevalent HPV types commonly associated with warts, (3) Also, to identify the role of HPV in skin and genital lesions, and its relation to p53 expression.

PATIENTS AND METHODS

This study was performed on 30 patients presented to the outpatient dermatology clinic of Kasr El-Eini Teaching Hospitals during the period from May, 2002 to April, 2004, with common and/or genital warts.

Skin biopsies:

Two skin specimens were obtained from each patient. The first (4-6mm) was taken from the selected warts and the second (3mm) was taken from the normal skin. Half of each specimen was fixed in 10% natural buffered formalin, paraffin-embedded, and examined by routine pathology. The other half of the lesion was kept at 80°C for DNA extraction and HPV detection.

Immunohistochemical staining for p53 detection:

Five micrometer sections were cut onto sialinized slides (positive charge, Optiplus, BioGenex, CA, USA), air-dried overnight at room temperature (RT), incubated at 55°C for 1h, dewaxed in xylene and rehydrated using graded alcohol concentrations. The antigen retrieval method was done by heating for 10min in 0.1mol/L citrate buffer (pH 6) in a microwave oven (600W). Slides were then incubated for 10min in 0.3% H2O2 to abolish endogenous peroxidase activity and the p53 monoclonal antibody (clone DO-7, Cal Bio Chem) at a working concentration (1:50) was added. Slides were then incubated for 2h at RT, washed and stained with the universal labeled streptavidin biotin method (Vector Laboratories, Peterborough, UK) according to the manufacturer’s instructions. Positive staining was detected with 0.3% 3,3’-diaminobinzidine tetrahydrochloride (DAB) in citrate buffer and nuclei were counterstained with Meyer’s hematoxylin. Negative controls were obtained by replacing the primary antibody by non-immunized rabbit or mouse serum. Immunoreactivity for p53 was classified as follows; negative, <10% positive cells; weak positive, 10-50% positive cells; moderately positive, >50% but <75% positive cells; and markedly positive, >75% positive cells, as shown in Figs. (1,2,3).

DNA extraction:

DNA extraction from 100mg of each specimen was performed according to standard protocols [12].
PCR for HPV DNA:

Broad-spectrum HPV-DNA amplification was performed using the short polymerase chain reaction (PCR) fragment (GP5 and GP6) primer set, which amplifies a 150bp fragment in the L1 region of the HPV genome. Primer sequences and PCR conditions were done as previously described [13]. Each experiment was performed with positive and negative PCR controls. To prevent PCR product carry over, dTTP was replaced by dUTP and uracil-N-glycosylase was added. The amplicons were run on a 3% agarose gel and the 150bp product was visualized with ethidium bromide staining. All HPV-negative cases were confirmed by the second PCR assay using standard DNA concentration, as well as a 10x diluted DNA sample to exclude the presence of PCR inhibitors. Appropriate positive and negative PCR controls were run with all reactions (Fig. 4).

Typing of human papillomavirus:

Samples scored positive for HPV-DNA were genotyped with the INNO-LiPA HPV prototype research assay (Inno-LiPA). The HPV-LiPA was performed essentially as described earlier for the hepatitis C virus INNO-LiPA [14]. Briefly, the labeled amplicon was allowed to hybridize and was mounted on a strip. After stringent washing, streptavidine labeled with alkaline phosphatase was used to trace the hybridized products and nitro blue tertazolium and 5-bromo-4-chloro-3-indolyl-phosphate were used as substrate according to the manufacturer’s instructions.
Statistical analysis:

The SPSS 11 program (Statistical Package for the Social Sciences) was used. Chi-square test (χ²) was performed to test the association variables for categorical data. Fisher exact test was done in table containing value less than 5. Student’s t-test was also used, and Level of significance was <0.05.

RESULTS

Clinical data:

Thirty patients complaining of common and genital warts were included in this study. These patients were divided into two groups: Group I involved 20 cases with common warts and group II involved 10 cases with genital warts. Among group I, 11 patients were males and 9 were females, with an average age of 24.2 years and a range from 11 to 46 years. Two patients had an associated itching, one patient had an associated pain, and another one had an associated palmar warts. Among group II, 8 were males and 2 were females, with an average age of 38.4 and a range from 21 to 65 years. Four patients had an associated itching, five had a bleeding on touch, and one case had vaginal discharge in both groups. The duration of warts ranged from one week to thirty years while their number ranged from single to multiple warts.

HPV DNA, its type, and P53 immunohistochemical staining:

Among patients with common warts (group I), 4/20 (20%) cases were positive for HPV DNA of types 11, 31, 6, and 33 (p=0.28). Also, four other patients (20%) had p53 expression (p=0.26), and no single patient showed positivity for both HPV DNA and p53. On the other hand, 6/10 (60%) patients of genital warts (group II) were positive for HPV DNA. Five cases were positive for HPV type 6 and one case was positive for HPV type 11, as shown in Table (1). Three patients of group II (30%) were positive for p53, and two of them (66%) were positive for both HPV and p53. In the normal skin specimens, 2/30 (6.6%) cases were positive for HPV which were of types 5, and 31. The difference however between the normal and the other groups was not statistically significant.

HPV DNA, type, and p53 in relation to number of warts:

The relationship between the number of warts in groups and HPV DNA, its type, and p53 was studied as shown in Table (2). Although 9 out of 10 positive for HPV DNA had multiple warts and the other case had only single wart, still this did not reach a statistically significant level. Also no significant difference was noticed between those with single versus multiple warts regarding HPV type or p53 staining.

Table (1): Reactivity of HPV, genotype and p53 in different types of warts.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Common wart N=20 Gr. I</th>
<th>Genital wart N=10 Gr. II</th>
<th>Normal controls N=30</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HPV DNA</strong></td>
<td>4 (20%)</td>
<td>6 (60%)</td>
<td>2 (6.6%)</td>
<td>0.280</td>
</tr>
<tr>
<td><strong>p53 reactivity:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–ve</td>
<td>16 (80%)</td>
<td>7 (70%)</td>
<td>30 (100%)</td>
<td></td>
</tr>
<tr>
<td>+ve</td>
<td>2 (10%)</td>
<td>3 (30%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>++++ve</td>
<td>2 (10%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.261</td>
</tr>
<tr>
<td><strong>HPV type:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 5</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (50%)</td>
<td></td>
</tr>
<tr>
<td>Type 6</td>
<td>1 (25%)</td>
<td>5 (83.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Type 11</td>
<td>1 (25%)</td>
<td>1 (16.6%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Type 31</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
<td>1 (50%)</td>
<td></td>
</tr>
<tr>
<td>Type 33</td>
<td>1 (25%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.188</td>
</tr>
</tbody>
</table>
DISCUSSION

HPVs are small DNA tumor viruses that induce cutaneous and mucosal proliferation of epithelial cells (papillomas, condylomas, and warts). To date, over 100 different types of HPVs have been identified, and all target epithelial tissues for infection \[15,16\]. Although most of HPV types cause benign lesions, other types (HPV16 and HPV18) are associated with malignancy, and are detected in almost all cases of cervical cancer \[17\]. Carcinogenesis is associated with cutaneous HPV infections in certain patients such as organ transplant recipients, and patients with EV who have an underlying immune system abnormality \[18\]. The present study was carried out to demonstrate the prevalence of HPV, and its types in relation to p53 expression in common and genital warts.

In our study, prevalence rate of HPV DNA was higher in patients with warts compared to low prevalence 2/30 (6.6%) of HPV infection in normal control specimens. This is in accordance with a previous study done by Iftner et al. \[20\] where very low prevalence (4.7%) of HPV infection were found in normal skin biopsies. However, in another study done by Astori et al. \[21\] on human papillomaviruses infection in normal skin of immunocompetent hosts, they found high prevalence of HPV infection. This difference could be either because all normal skin biopsies were carefully cleaned with disinfectant before they were taken, which may have reduced the observed high skin surface contamination with virus particles \[22\] or due to the circumstance that earlier studies used skin biopsies from unusual anatomical sites such as eyelids as control samples.

Regarding the different HPV genotypes found in the present study, four distinct HPV types were found in common warts which were HPV-11 (25%), HPV-31 (25%), HPV-6 (25%), and HPV-33 (25%), and two distinct HPV types were found in genital warts which were HPV-6 (83.3%), and HPV-11 (16.6%), compared to HPV-5 (50%), and HPV-31 (50%) that were detected in normal skin biopsies. HPV type 6 was the predominant genotype in genital warts. Similar results were reported in a previous study.

Table (2): Correlation between number of warts and HPV DNA, Type, and P53 expression.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of warts</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
<td>Multiple</td>
</tr>
<tr>
<td><strong>HPV DNA:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n=10)</td>
<td>1 (10%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>Negative (n=20)</td>
<td>3 (15%)</td>
<td>17 (85%)</td>
</tr>
<tr>
<td>Total (n=30)</td>
<td>4 (13.3%)</td>
<td>26 (86%)</td>
</tr>
<tr>
<td><strong>HPV Type:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 6 (n=6)</td>
<td>1 (16.7%)</td>
<td>5 (83.3%)</td>
</tr>
<tr>
<td>Type 11 (n=2)</td>
<td>0 (0%)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Type 31 (n=1)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Type 33 (n=1)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Total (n=10)</td>
<td>1 (10%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td><strong>P53 staining:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–ve (n=23)</td>
<td>3 (13%)</td>
<td>20 (87%)</td>
</tr>
<tr>
<td>+ve (n=5)</td>
<td>1 (20%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>++++ve (n=2)</td>
<td>0 (0%)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>Total (n=30)</td>
<td>4 (13.3%)</td>
<td>26 (86.7%)</td>
</tr>
</tbody>
</table>

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done by Tendler et al. [23] on 12 cases of condyloma acuminatum (CA). They found that 7/12 (58%) cases were positive for HPV, five of them were positive for HPV type 6. Also, another study done by Moscicki [24] on HPV infection in adolescent populations supported this finding where he stated that low-risk HPV types (HPV 6 or 11) were associated with the development of genital warts. However, in a study done by Daneshpouy et al. [25] on three patients with CA, they found one patient positive for HPV type 18. This may be attributed to the relatively small number of the patients involved in their study.

To study the association between p53 expression and HPV in common and genital warts, 4/20 (20%) of cases of common warts and 3/10 (30%) among the patients of genital warts were positive for p53 expression. This is in agreement with a previous study done by Castern et al. [26] on 21 patients with CA, where 13 cases were positive for p53. Our result is also supported by Zhang et al. [27] who studied 21 patients with CA, and found 5/21 (24%) of cases showing p53 over expression. However, in a study done by Daneshpouy et al. [25] on three patients with CA, they found no p53 positive cases, and this may be due to the relatively small number of the patients involved in that study.

Our results showed no single case positive for both HPV and p53 in common warts. However, in genital warts, there were 2/10 (20%) cases that were positive for HPV type 11 and 6 and p53 expression. Ranki et al. [28] supported our finding where they did not find any association between HPV type and p53 expression in common warts. On the other hand, Lassus and Ranki [29] found an abnormal p53 expression in skin lesions infected by HPV, and suggested that p53 was susceptible to aberrations in HPV infected cells. The same result was also obtained by Arany et al. [30] who found that the presence of HPV can diminish p53. Our findings are supported by a previous study done by Castren et al. [26] who found no correlation between p53 protein expression and HPV status, and they suggested that degradation of p53 by HPV is not a major mechanism in HPV-induced cell proliferation. Our findings are also in agreement with Giannoudis et al. [31] who found no significant relationship between the frequency of p53 expression and either HPV type or lesion grade. HPV express factor E6 that provokes p53 degradation via an ubiquitin-dependent pathway, hindering the apoptosis in the host cell [32]. E6 binds to p53 in a trimeric complex with an ubiquitin ligase called E6AP [33,34]. Formation of this complex results in the ubiquitination of p53 and subsequent degradation by the 26S proteasome, leading to a reduction in the half-life of p53 from several hours to less than 20min in keratinocytes. E6 can also indirectly down-regulate p53 activity through its association with p300/CBP, which is a coactivator of p53 [38,39,40]. Since p53 regulates both the G1/S and G2/M checkpoints of the cell cycle, its rapid turnover results in abrogation of these controls, leading to chromosomal duplications and centrosomal abnormalities [41,42].

From our results, we can conclude that there is no definite relation between p53 expression, and the reactivity of HPV infection or HPV types. Also, Prevalence rate of HPV infection in warts is higher than those of normal control group, and Egyptian patients with genital warts had higher prevalence rate of HPV than those with common warts. In Egypt, HPV types 6, and 11 are the most prevalent genotypes associated with genital warts and HPV types 6, 11, 31, and 33 are associated with common warts. So, further studies are needed to clarify other mechanisms, rather than p53 inhibition, by which HPV may induce cell proliferation.

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