Histopathologic and immunohistochemical distinction of condyloma and seborrheic keratosis in the genitofemoral area

Yu-Hung Wu1,2,4,*, Pa-Fan Hsiao1,2,4, Chi-Kuan Chen1,3,4

1 Mackay Medical College, New Taipei City, Taiwan
2 Department of Dermatology, Mackay Memorial Hospital, Taipei, Taiwan
3 Department of Pathology, Mackay Memorial Hospital, Taipei, Taiwan
4 Mackay Medicine, Nursing and Management College, Taipei, Taiwan

Article history:
Received: Mar 6, 2012
Revised: Sep 18, 2012
Accepted: Oct 1, 2012

Keywords:
condyloma
genitofemoral area
human papillomavirus
Ki-67
p21
seborrheic keratosis

A B S T R A C T

Background: Making a clinical and histological distinction between condyloma and seborrheic keratosis in the genitofemoral area can be difficult. This study aimed to find reliable histological and immunohistological criteria to diagnose these entities.

Methods: We retrospectively studied genitofemoral skin biopsy specimens obtained between January 2004 and December 2007 that had been diagnosed as showing condyloma or seborrheic keratosis. The histological findings were assessed and immunohistochemical stains were performed for human papillomavirus, Ki-67, and p21. DNA was extracted from paraffin sections and amplified by polymerase chain reaction to detect the presence and type of human papillomavirus.

Results: DNA extraction was successfully performed for 58 lesions. The final diagnoses were condyloma in 41 and seborrheic keratosis in 17. The diagnosis of condyloma rather than seborrheic keratosis was likely in the presence of broad, evenly distributed reticulated acanthosis \( p < 0.0001 \), koilocytosis \( p < 0.001 \), a fascicular arrangement of keratinocytes \( p < 0.01 \), and an absence of horn cysts \( p < 0.01 \). Immunohistochemical staining supported the diagnosis of condyloma when positive for human papillomavirus \( p < 0.0001 \), Ki-67 \( p < 0.0001 \), and p21 \( p < 0.0001 \).

Conclusion: A combination of histological and immunohistochemical findings is useful to distinguish condyloma from seborrheic keratosis in the genitofemoral area.

Copyright © 2012, Taiwanese Dermatological Association. Published by Elsevier Taiwan LLC. All rights reserved.

INTRODUCTION

Polypoid or verrucous lesions in the genitofemoral area may be harmless seborrheic keratoses or contagious condyloma acuminata. A solitary lesion is particularly difficult to diagnose clinically, requiring pathological evaluation. A condyloma is an epithelial hyperplasia induced by infections with human papillomavirus (HPV). The histological gold standard for the diagnosis of condyloma is the presence of koilocytosis1; however, it is not present in every lesion, and the histological diagnosis may be inaccurate.2,3 Therefore, the best way to diagnose a condyloma is to demonstrate the presence of virus in the lesion, especially the most common HPV types 6 and 11.4,5

In the 1990s, several methods were developed for the detection of viral DNA. Articles were published describing genital seborrheic keratosis associated with HPV in up to 50% of cases.6,7 This raised the question of how many lesions diagnosed as genitofemoral seborrheic keratosis were in fact condyloma.2 These virologic tests, whether using in situ hybridization or the polymerase chain reaction (PCR), can accurately detect HPV DNA.7,9 However, test results must be correlated with clinical characteristics, pathologic characteristics, and HPV type to avoid false-positive diagnoses.10 Meanwhile, DNA extraction is not feasible in every case. We therefore decided to revisit the histological and immunohistochemical features that could reliably distinguish between condyloma and genitofemoral seborrheic keratosis.

* Corresponding author. Department of Dermatology, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Road, Taipei 10449, Taiwan. Tel.: +886 2 25433535.
E-mail address: yuhung_wu@yahoo.com (Y.-H. Wu).
The formalin-fixed, paraffin-embedded tissues were stained with hematoxylin and eosin and specific stains for HPV, Ki-67, and p21WAF1/Cip1. Standard microscopic pathology examination was performed with appropriate controls. The slides were numbered and read independently initially by one dermatopathologist (Y.H.W.) and one pathologist (C.K.C.) who had been blinded to the previously recorded clinical, pathologic, and virologic diagnoses. Histopathological findings were recorded according to the definitions listed in Table 1. Any disagreement between the observers was resolved by reviewing the slides together later.

### Material and methods

#### Patient selection

From January 2004 to December 2007, we retrieved from our dermatopathology database the records of all patients who had had a skin biopsy diagnosis of either condyloma or seborrheic keratinosis in the genitofemoral area. The Institutional Review Board approved this study using the tissue specimens from these cases (MMH-I-S-357).

#### Histopathological examination

The formalin-fixed, paraffin-embedded tissues were stained with hematoxylin and eosin and specific stains for HPV, Ki-67, and p21WAF1/Cip1. Standard microscopic pathology examination was performed with appropriate controls. The slides were numbered and read independently initially by one dermatopathologist (Y.H.W.) and one pathologist (C.K.C.) who had been blinded to the previously recorded clinical, pathologic, and virologic diagnoses. Histopathological findings were recorded according to the definitions listed in Table 1. Any disagreement between the observers was resolved by reviewing the slides together later.

#### Immunohistochemistry

##### Anti-HPV antibody

The monoclonal mouse anti-HPV antibody (clone K1H8, DakoCarpinteria, CA, USA) that was used is immunoreactive to HPV types 6, 11, 16, 18, 31, 33, 42, 51, 52, 56, and 58, with immunostaining largely confined to the granular cell layer nuclei. The number of positive cells per high-power field, (400×) was graded as + (2–10 cells), ++ (11–20 cells), or +++ (>20 positive cells).

##### Ki-67 antigen

The monoclonal mouse anti-human Ki-67 antigen, (clone MIB-1, DakoCytomation, Copenhagen, Denmark), was used, examining for positive immunostaining in the basal cell layer of the epidermis, which is present in normal tissue and was used as an internal control. Nuclear staining in the spinous layer indicates abnormal active keratinocyte proliferation above the basal cell layer. When present, this was graded based on the percentage of positive cells as + (10–25%), ++ (25–50%), or +++ (>50% positive cells).

##### Anti-human p21 antibody

The monoclonal mouse anti-human p21 (clone SX118; DakoCytomation, Copenhagen, Denmark), was used, examining for positive immunostaining in the basal cell layer of the epidermis which is present in normal tissue and was used as an internal control. Nuclear staining in the spinous layer indicates abnormal active keratinocyte proliferation above the basal cell layer. When present, this was graded based on the percentage of positive cells as + (10–25%), ++ (25–50%), or +++ (>50% positive cells).

#### HPV DNA extraction and sequencing

Four or five 8-μm sections were cut from each paraffin-embedded block. DNA was obtained using the QIAamp DNA FFPE tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer’s instructions. PCR with the primer pair FAP59/FAP64 was used to detect HPV DNA as previously described.9,10 The HPV DNA products from PCR spanned the L1 region from nucleotides 6044 to 8248 and were extracted from the tissue, and DNA was obtained using the QIAamp DNA FFPE tissue kit (Qiagen). DNA was obtained using the QIAamp DNA FFPE tissue kit (Qiagen). DNA was obtained using the QIAamp DNA FFPE tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer’s instructions. PCR with the primer pair FAP59/FAP64 was used to detect HPV DNA as previously described.9,10 The HPV DNA products from PCR spanned the L1 region from nucleotides 6044–8248.10 HPV types were determined by sequence analysis on an ABI Prism 377 DNA sequencer (Perkin-Elmer, Fremont, CA, USA). Comparison of the DNA sequences obtained with those of previously established HPV types and putative types were performed by using the BLAST server (http://www.ncbi.nlm.nih.gov/blast/).

#### Diagnostic criteria for condyloma

When HPV type 6, type 11, and other subtypes that have been reported to be associated with condyloma or seborrheic keratinosis in the genitofemoral area were extracted from the tissue, a final diagnosis of condyloma was made. For specimens without HPV DNA or with a type of HPV DNA not known to be associated with condyloma, the final diagnosis was made based on

### Table 1 Definition of histological findings.

<table>
<thead>
<tr>
<th>Finding (presence of)</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cauliflower</td>
<td>A papilloid lesion with prominent papillomatosis</td>
</tr>
<tr>
<td>Broad reticulated acanthosis</td>
<td>The width of the suprabasal spinous layer in the reticulated epidermal proliferation was greater than five keratinocytes and the reticulated pattern is evenly present in more than half of the lesion</td>
</tr>
<tr>
<td>Koilocytosis</td>
<td>The presence of a perinuclear halo in the granular cell layer</td>
</tr>
<tr>
<td>Fassiculor arrangement of keratinocytes</td>
<td>The spindle-shaped keratinocytes are arranged in a fascicular pattern</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>The presence of pleomorphic or hyperchromatic nuclei and scattered dyskeratosis throughout the epidermis not severe enough for the diagnoses of bowenoid papulosis or squamous cell carcinoma in situ</td>
</tr>
<tr>
<td>Horn cysts</td>
<td>Well-differentiated small intraepidermal keratinous cysts with a granular cell layer</td>
</tr>
<tr>
<td>Hyperpigmentation</td>
<td>Hyperpigmentation of the keratinocytes compared to peripheral normal skin</td>
</tr>
<tr>
<td>Spongiosis</td>
<td>Intercellular edema and widening of the intercellular space in the epidermis</td>
</tr>
</tbody>
</table>
all the information available, including clinical appearance, pathologic findings, and immunohistochemical staining.

**Statistical analysis**

The histological features of condyloma and seborrheic keratosis were compared using Fisher’s exact test. A value of $p < 0.05$ was considered statistically significant in the differences between two groups.

**Results**

A total of 64 patients with 67 lesions involving the genitalia, anal, or perianal area, pubic area, groin, inner thigh, lower abdomen near the pubis, or buttocks near the intergluteal cleft were retrieved (Table 2). The findings from the hematoxylin and eosin slides and immunohistochemical stains are summarized in Table 3.

**Diagnosis confirmation**

DNA extraction failed in nine specimens, which were excluded from analysis, leaving 58 specimens in the study. HPV DNA was present in 40, including HPV type 4 (2 specimens), type 6 (29), type 11 (6), type 32 (2), and type 43 (1). Type 4 HPV, which typically causes verruca plantaris, was thought to be a contaminant. The two specimens with that type had typical pathologic features of seborrheic keratosis. No koilocytosis was present, although one specimen did have a weak reaction to p21. However, after correlation of all the clinical and pathological findings, the two specimens were diagnosed as seborrheic keratosis. The two specimens with type 32 and the one specimen with type 43 were diagnosed as condyloma based on the high association with mucosal epithelial hyperplasia reported previously.12,13 The distribution of the HPV genotype is similar to that in a recent report of genital warts in Taiwan.14 Among the 18 specimens with no HPV DNA, three were classified as condyloma based on prominent koilocytosis or positive HPV staining. The final diagnoses thus included 41 condylomas and 17 seborrheic keratoses.

**Comparison between two groups**

**Clinical comparison**

The average age of patients with condyloma (31 years) was younger than those with seborrheic keratosis (54 years). Condylomas were more common in males (32) than females (9), whereas the gender distribution was closer to equality in seborrheic keratosis (7 males and 10 females). Lesions occurring on the scrotum, penis, vulva, and perineum were more likely to be condyloma, although seborrheic keratosis could be present in any of those areas (Table 2).

**Histopathologic findings in two groups**

Condylomas and seborrheic keratosis may both have a reticular architecture. The rete ridges were significantly more likely to be broad, with a similar width in condyloma (Figure 1A) compared to a variable size in seborrheic keratosis ($p < 0.01$) (Table 3). Koilocytosis was present in 68% (28/41) of condylomas (Figure 1B), but can occasionally be observed in seborrheic keratosis (3/17, 18%; $p < 0.01$). Keratinocytes in condylomas were often arranged in tightly interwoven fascicles (Figure 1C), a finding rarely present in seborrheic keratosis ($p = 0.01$). Horn cysts were frequently seen in seborrheic keratosis but are not common in condylomas ($p < 0.01$). The presence of a cauliflower shape, pigmentation, papillomatosis, spongiosis, and dermal inflammation did not differ significantly between the two disorders.

**Immunohistochemical stains in two groups**

Immunohistochemical staining (HPV, Ki-67, and p21) results differed significantly between condyloma and seborrheic keratosis (all $p < 0.01$; Table 3). The HPV stain was specific but not sensitive. Only 20% of condylomas (8/41) had a strong positive result (+++ or ++++), and most of those eight lesions could be easily diagnosed.

---

**Figure 1** Important diagnostic features of condyloma. (A) Evenly distributed broad reticulated acanthosis and cauliflower architecture (H&E stain, 40×). (B) Koilocytosis of the granular cell layer. (C) Fascicular arrangement of the keratinocytes (H&E stain, B, C 200×). (D) Coexistence of both koilocytosis and a fascicular arrangement of keratinocytes.
histopathologically. Conversely, Ki-67 and p21 were not specific but were very sensitive for the diagnosis of condyloma, with more than 80% of condylomas positive for at least one of the stains. Nearly half (46%, 19/41 for Ki-67; 49%, 21/41 for p21) of the specimens had a strongly positive result (+++ or +++) (Figure 2).

**Sensitivity, specificity, predictive value, and likelihood ratio in two groups**

Seven features—koilocytosis, broad reticulated acanthosis, a fascicular arrangement of keratinocytes, horn cysts, HPV-positive staining, Ki67-positive staining, and p21 positivity—differed significantly between the two types of lesion (Table 3). The characteristics of the various diagnostic criteria are shown in Table 4. Based on likelihood ratios, the most helpful findings to rule in the diagnosis of condyloma (positive likelihood ratio ≥ 3) were the presence of koilocytosis, a fascicular arrangement, and positive staining for HPV, Ki-67, and p21. The most helpful findings to rule out condyloma (negative likelihood ratio ≤ 0.2) were an absence of broad reticulated acanthosis and negative Ki-67 and p21 staining.

### Table 4 Diagnostic characteristics of various features in evaluating condyloma in the genitofemoral area.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>PPV, %</th>
<th>NPV, %</th>
<th>LR+</th>
<th>LR−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koilocytosis</td>
<td>68</td>
<td>82</td>
<td>90</td>
<td>52</td>
<td>3.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Broad reticulated acanthosis</td>
<td>98</td>
<td>59</td>
<td>85</td>
<td>91</td>
<td>2.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Fascicular arrangement</td>
<td>46</td>
<td>94</td>
<td>95</td>
<td>42</td>
<td>7.9</td>
<td>0.6</td>
</tr>
<tr>
<td>Horn cysts</td>
<td>29</td>
<td>29</td>
<td>50</td>
<td>15</td>
<td>0.4</td>
<td>2.4</td>
</tr>
<tr>
<td>HPV-positive staining</td>
<td>63</td>
<td>100</td>
<td>100</td>
<td>53</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Ki-67-positive staining</td>
<td>88</td>
<td>71</td>
<td>88</td>
<td>71</td>
<td>3.0</td>
<td>7.1</td>
</tr>
<tr>
<td>p21-positive staining</td>
<td>83</td>
<td>94</td>
<td>97</td>
<td>70</td>
<td>14.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

LR+ = positive likelihood ratio; LR− = negative likelihood ratio; NPV = negative predictive value; PPV = positive predictive value.

* Divided by zero.
Discussion

This study examined the reliability of old criteria and provided new information on the distinction between condyloma and seborrheic keratosis in the genitofemoral area. In low-power fields, condyloma often had broad and evenly distributed reticulated acanthosis, a finding that was more sensitive and useful than cauliflower or papillomatous architecture. In higher magnification, about two-thirds of condylomas had diagnostic koilocytosis. However, there are two pitfalls which clinicians should be aware of when interpreting the histopathologic findings. First, koilocytes can be occasionally seen in seborrheic keratosis; second, horn cysts, a very common finding usually indicating seborrheic keratosis, may occasionally appear in a condyloma.

A very helpful sign that has not previously been reported is the fascicular arrangement of keratinocytes, found in half the condylomas in our series but infrequently in seborrheic keratosis. In the authors’ personal experience, this pattern may only appear occasionally in nevus sebaceous. One possible explanation for this peculiar arrangement in condylomas is that the disorder involves squamous keratinocyte proliferation. Seborrheic keratosis, on the other hand, involves the proliferation of both squamous and basoloid cells, with the latter often predominating. Therefore, a fascicular arrangement of keratinocytes would not be common in seborrheic keratosis.

For lesions that cannot easily be diagnosed using histopathological features, immunohistochemical stains for Ki-67 and p21 were helpful. The most frequently used immunohistochemical stains in the diagnosis of HPV-associated genital intraepithelial neoplasia are those for Ki-67 and p21. Condylomas have been demonstrated to be a proliferative keratinocytic lesion with Ki-67 expression, however, Ki-67 staining was normally present in the basal cell layer of all specimens, and interpretation required skill to distinguish normal from abnormal staining. p16 is a cell-cycle regulatory protein overexpressed in cell nuclei infected by high-risk HPV.11,15 It has been found that p16 expression is not helpful with vulvar lesions associated with low-risk HPV infection, including condylomas.9

In recent research, another cell-cycle control protein, p21, was noted to be produced in cells infected with low-risk HPV types.11 Our study demonstrated a similar result. p21 is a cyclin-dependent kinase inhibitor that usually results in G1-phase cell-cycle arrest.11,15 One would expect p21 to be not expressed in the proliferation of HPV-infected keratinocytes. In contrast, increased p21 expression has been found in the suprabasal cells of condylomas, and this was confirmed in our study. HPV-infected keratinocytes expressing p21 can still proliferate, as shown by the co-expression of p21 and Ki-67 studies, might be attributed to host cell reaction. The findings are very useful in the diagnosis of condyloma because cells in normal epithelium do not show a concurrent expression of both positive and negative regulatory proteins. Moreover, p21 nuclear staining was present in the upper epidermis without basal cell positivity, which is easier to read compared to Ki-67 staining.

The primary limitation of this investigation was the small number of specimens available for study. In addition, bias was possible between different observers who made the diagnosis of the lesions. A greater variety of lesions, including resolving condylomas for example, should be examined to confirm the results. In particular, a variety of proliferative epidermal lesions should be evaluated to see if the fascicular arrangement of keratinocytes is a valid histological marker for HPV infection. The expression of cell-cycle proteins in various subtypes of HPV infection also needs to be more fully understood.

In conclusion, broad reticulated acanthosis and a fascicular arrangement of keratinocytes are helpful findings at scanning magnification that raise the possibility of condyloma. If these patterns are present, high-power fields should be carefully examined for koilocytosis. If the diagnosis is still in question, staining with Ki-67 and p21 may provide indirect evidence of HPV infection.

Acknowledgments

We thank Mr Po-Tsang Chen and Mr Schu-Rern Chern (Department of Medical Research, Mackay Memorial Hospital) and Dr Chih-Ping Chen (Department of Gynecology, Mackay Memorial Hospital) for their help with HPV DNA extraction and sequencing. This work was supported by grants from the Mackay Memorial Hospital MMH-E-9730.

References