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Original Contribution

The molecular-based differentiation of Heck's disease from its mimics including oral condyloma and white sponge nevus

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ABSTRACT

Heck's disease (focal or multifocal epithelial hyperplasia) is a benign, rare condition of the skin and mucous membranes induced by human papillomavirus (HPV) infection. Other entities that can induce large papillomatous lesions that involve the mucous membranes and skin include condyloma acuminatum, which is sexually transmitted, and white sponge nevus, often due to a mutation of cytokeratin 4 or 13. Six cases diagnosed as either Heck's disease (n = 2) or white sponge nevus (n = 4) and 6 oral condyloma were compared on histologic grounds and analyzed in situ for HPV DNA, including HPVs 6,11, and 13, as well as cytokeratins 4 and 13. Each case showed marked acanthosis, and para/hyperkeratosis. More variable histologic findings included rete ridge elongation, keratinocyte degeneration, and perinuclear halos. High copy HPV 13 DNA was evident in the squamous cells towards the surface in the two cases diagnosed as Heck's disease and in two cases diagnosed as white sponge nevus on clinical grounds. HPV 6/11 was found in each of the six condyloma. Marked decrease in either cytokeratin 4 or 13 was evident in the two cases diagnosed as white sponge nevus that were HPV DNA negative. It is concluded that in situ hybridization analyses including HPVs 6, 11, and 13 as well as immunohistochemistry for cytokeratins 4 and 13 can differentiate Heck's disease from condyloma and white sponge nevus, which can be difficult to differentiate on clinical and histologic grounds.

1. Introduction

Heck's disease, also known as focal or multifocal epithelial hyperplasia, is a benign, rare condition marked by squamous cell proliferations of the oral mucosa or skin due to human papillomavirus (HPV) infection. HPVs 13 and 32 are primarily implicated, but other subtypes have also been reported [1,2]. HPVs 13 and 32 are among the low risk HPV types such as 2, 6, 11, as opposed to the high risk HPV types associated with malignancy such as 16, 18, 31, 51 and others.

Heck's disease manifests as multiple, round, asymptomatic sessile or exophytic papules or nodules in the oral mucosa often 5–10 mm in diameter [1,3]. The lesions can be classified into two clinical variants. The papulonodular subtype, found on buccal and labial mucosa, palate, and oral cavity commissures, appears pink and smooth, and is predominant in young patients [1,3-5]. The papillomatous subtype occurs on the lingual and gingival tissues, appears white with cobblestoning, and occurs more often in adult patients [1,3-5]. The disease course is one of spontaneous remission and recurrences [6]. Although benign, many patients still receive treatment for aesthetic reasons [7].

Heck's disease is most prevalent in children with 97% of patients (n-110) being children in one study [6,7]. It has been postulated that the immature immune system is unable to clear the viral infection [8]. Because the lesions resemble oral condyloma, ruling out sexual abuse is a key component of the workup in children [9]. Immunocompromised patients are also at an increased risk. The disease has a strong predilection for females, with ratios as high as 5 to 1 [1]. An increased prevalence in low socioeconomic communities has also been described [6]. Heck's disease occurs at increased rates among Eskimos, Native Americans and Latino Americans [10-12].

It has been hypothesized that there is a genetic predisposition for increased susceptibility to HPV 13 or 32 infection [13,14]. Alternatively, horizontal transmission via contaminated food/eating utensils in the context of poor hygiene among low socioeconomic groups has also been proposed [15]. This may explain the preference of HPVs 13 and 32 for the oral cavity, which is a rare HPV site except for the sexually spread HPV types 6, 11, and 16.

White sponge nevus is a genetic disease often due to mutations of keratin 4 and/or 13 [16,17]. According to Liu et al. [18], all patients

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with family history exhibit a keratin 4 or 13 mutation. However, the sporadic patients with no familial background were heterogeneous: the majority of them (4/5) did not exhibit any mutational evidence, [19] and thus they may have had other etiologies. Indeed, HPV 16 DNA sequences were detected in biopsy of oral white sponge nevus by Cox et al. [20]. Another report concluded that the thickening of superficial layer is due to dysfunction of Odland bodies (keratinosomes) involved in the normal desquamation of the skin; hence these lesions usually present as large exophytic lesions in the oral cavity [21]. Oral condylomata, much like the more common genital tract condyloma acuminatum, are sexually transmitted papillary lesions due to infection by HPV 6 or 11 [22].

The purpose of this manuscript is to report on the histologic and molecular findings in 6 cases diagnosed as either Heck's disease or white sponge nevus as well as 6 oral condylomata in order to elucidate how to differentiate these lesions from each other.

2. Materials and methods

2.1. Case selection

Formalin fixed, paraffin embedded tissues were available from 12 patients with oral papillomatous lesions. Six oral condylomata were included in the study. These cases were obtained from three women and three men with an age range of 25 to 49 years of age. The condylomata were present either in the oral cavity or at the lateral aspect of the lip/oral cavity junction and ranged in size from 8 to 13 mm. The diagnosis of condyloma was made after detection of HPV 6/11 DNA by in situ hybridization in combination with the histologic findings that included marked acanthosis and prominent perinuclear halos; two of the women and one man also had genital tract condylomata. The other six cases of oral exophytic lesions were identified based on clinical evaluation as either Heck's disease (n-2) or white sponge nevus (n = 4). The two cases of Heck's disease included a 59-year-old healthy male with an enlarged tongue and scalloped edge for 3 years, as well as a lip lesion in a 23-year-old Hispanic woman referred for evaluation. The four cases diagnosed as white sponge nevus on clinical grounds included a 32-year-old healthy female with extensive white hyperkeratotic buccal mucosal lesions, with a history of similar lesions 9 years prior that were misdiagnosed as thrush. The others were a 34 year old male with a “spongy” leukoplakic lesion on his buccal mucosa, a 37 year old female with diffuse white lesions on the right and left buccal mucosa which the patient said had been present as long as she can remember, and a 57 year old female with what was described as “leukoplakia” of the corner of the lip.

Histologically normal skin/lip mucosa in three aged matched controls was used as negative controls. Three cases of Bowen's disease of the vulva, each HPV 16 positive, served as positive controls for the HPV in situ hybridization and the immunohistochemistry testing.

2.2. In situ hybridization

HPV DNA in situ hybridization was done with a variety of individual biotin tagged probes that can detect HPV types 1, 2, 5, 7, 8, 13, and 57, as previously reported [23-25]. These HPV types are associated with skin but not genital tract lesions. The probes for HPV 5, 8, 13, and 57 were gifts from Dr. Ethel-Michele de Villiers. Also used was the HPV “genital tract high risk consensus probe” from Enzo Life Sciences (Farmingdale, NY) that can detect over 20 different HPV types including HPVs 16, 18, 30, 31, 33, 35, 45, 51, 58, 70, and the probe for “low risk” HPVs 6 and 11 from the same company. In brief, after protease digestion, the genomic HPV probes labeled with biotin were co-denatured with the tissue DNA, hybridized for 15 h, washed at intermediate stringency, and then detected using the HPV in situ hybridization kit from Enzo. The chromogens nitro-blue tetrazolium and 5-bromo-4-chloro-3′-indolyphosphate yield a blue signal due to the action of alkaline phosphatase that is conjugated to streptavidin with nuclear fast red as the counterstain.

2.3. Immunohistochemistry

Our immunohistochemistry protocol has been previously published [23-27]. The biopsies were tested for the following antigens: Ki67, p16, importin-β, exportin-5, (ABCAM, Cambridge MA), and Mcl1 (Enzo Life Sciences, Farmingdale NY) as well as cytokeratin 4 and 13 (Proteintech, Rosemont IL). The analyses were done on the automated Leica Bond platform with the modification that the Enzo Life Sciences peroxidase anti-mouse/rabbit conjugate was used instead of the Leica HRP polymer (catalogue # ADI-950-113-0100) as this reduced background [27].

3. Results

3.1. Histologic analyses

The hematoxylin and eosin stains of the twelve cases were examined blinded to the clinical diagnosis. Representative examples of the three different categories (oral condyloma, Heck's disease and white sponge nevus) are provided in Fig. 1. The oral condylomata showed marked acanthosis and variable amounts of para- and hyperkeratosis (Fig. 1A). Also note the many perinuclear halos that were most prominent in the epithelial crevices of the broad acanthotic bands of cells. The cases diagnosed on clinical grounds as Heck's disease also showed marked acanthosis, variable papillomatosis, as well as para- and hyperkeratosis, rete ridge elongation, as well as perinuclear halos that also were most prominent in the cells located in the epithelial crevices (Fig. 1B, C). The four cases diagnosed on clinical grounds as white sponge nevus showed very different histology. Two of the cases showed marked acanthosis and para/hyperkeratosis but, unlike the condylomata or two cases of Heck's disease, there was also prominent spongiosis as well as marked keratinocyte degeneration (Fig. 1D–F). The other two cases diagnosed as white sponge nevus did have marked acanthosis with para/hyperkeratosis but showed many squamous cells with perinuclear halos and less prominent spongiosis (Fig. 1G–I).

3.2. In situ HPV DNA analyses

Each case was tested for HPV DNA using probe cocktails for low risk genital tract HPVs (HPV 6/11), high risk genital HPVs (including HPVs 16, 18, 31, 33, 35, 51, and others) and for non-genital HPV types (including HPVs 1, 2, 5, 7, 8, 13, and 57). Each of the six oral condyloma was strongly positive for HPVs 6/11 (Fig. 2A). Each of the two cases diagnosed as Heck's disease showed a strong signal for the non-genital HPV types. Further analysis with the individual probes documented that the two cases of Heck's disease were HPV 13 positive (Fig. 2C, D, and E). Note that the hybridization signal for HPV 13 was intense in the cells towards the apical aspect of the lesion, and was present in cells with perinuclear halos and, thus equivalent to the results for HPVs 6/11 in the oral condylomata. Also, such a finding is indicative of robust viral replication and, probably, infectious virions as reported with productive infection by HPV at other sites [24]. Two of the cases diagnosed as white sponge nevus on clinical grounds were negative for HPV DNA (Fig. 2F). However, the other two cases diagnosed as white sponge nevus were each strongly positive for HPV DNA with the skin HPV probe cocktail; further testing documented that each of these lesions contained HPV 13 DNA in high copy numbers (Fig. 2G and H).

3.3. Immunohistochemistry analyses

Next, each case was tested for cytokeratin 4 and cytokeratin 13 blinded to the HPV DNA in situ hybridization data. Representative data is provided in Fig. 3. Note that the HPV infected squamous cells in the

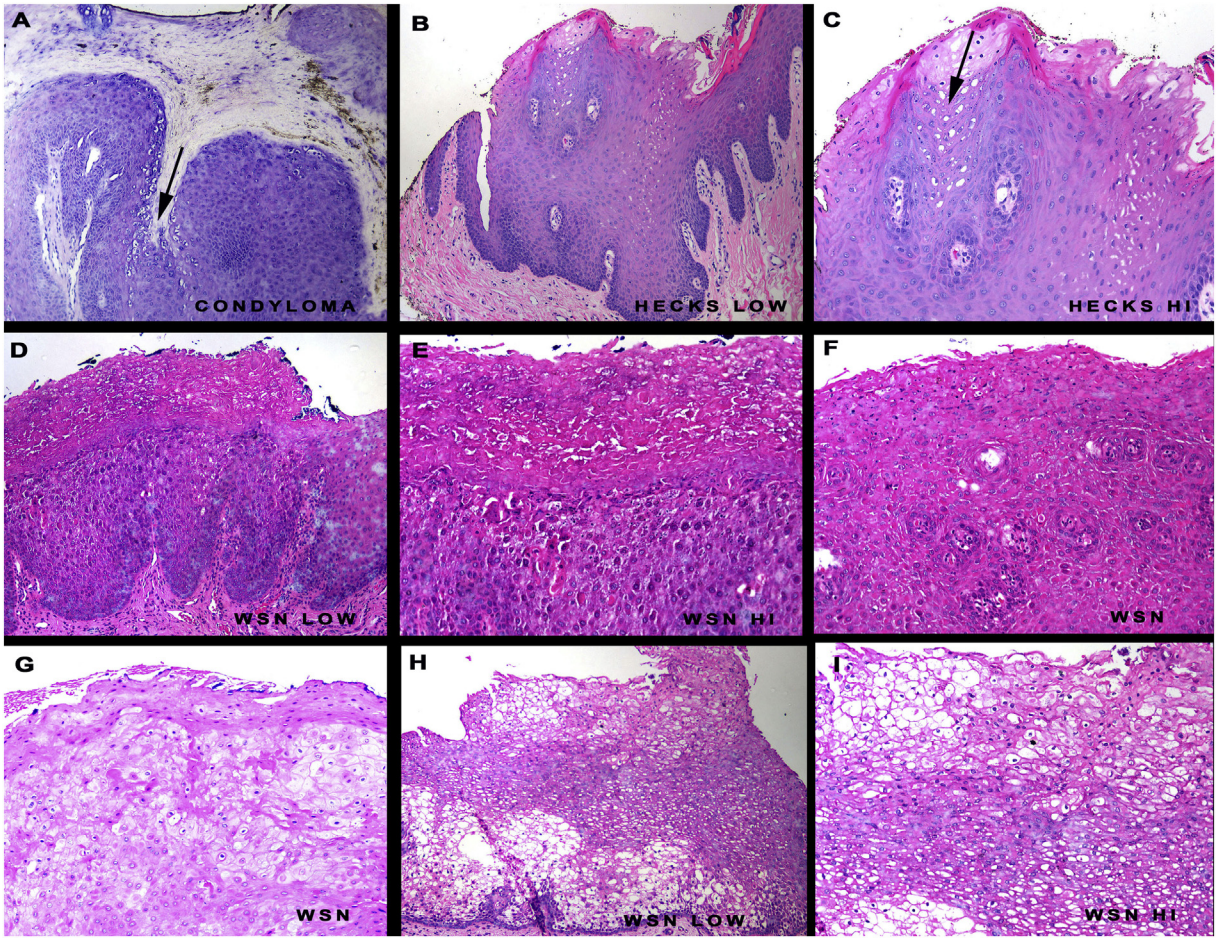

Fig. 1. Histologic correlates of oral condylomata, Heck's disease, and white sponge nevus. Panel A shows the marked acanthosis, para/hyperkeratosis, and prominent perinuclear halos (arrow) typical of oral condylomata. Similar histologic findings are noted in Heck's disease at low and high magnification (panels B and C). Note that the perinuclear halos are often in epithelial crevices (arrow). Panels D/E and F show two cases diagnosed on clinical grounds as white sponge nevus. Note the marked acanthosis and para/hyperkeratosis but also the diffuse spongiosis and degenerated keratinocytes. Panels G and H/I show the two other lesions diagnosed on clinical grounds as white sponge nevus. Note that perinuclear halos are more prominent in these lesions and there is little spongiosis.

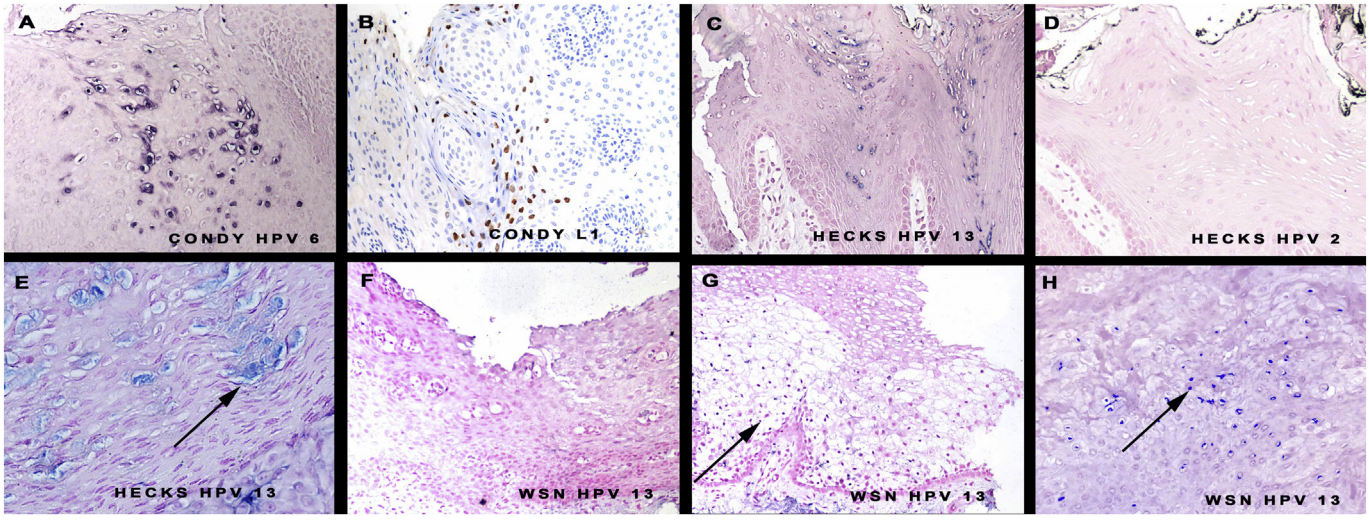

Fig. 2. HPV DNA detection in oral condylomata, Heck's disease, and white sponge nevus. Panel A shows the strong signal for HPV 6/11 in the oral condylomata. This viral number is usually associated with infectious virions as evident by the strong signal for the L1 capsid protein (panel B). Panel C shows a similar distribution for the signal for HPV 13 in a case of Heck's disease with a negative result for HPV 2 DNA (panel D). Panel E shows the HPV 13 signal for the other case diagnosed as Heck's disease; note that the positive cells are in cells with perinuclear halos (arrow). Panel F shows the lack of signal for HPV 13 in a white sponge nevus (panels D/E in Fig. 1). However, panels G and H show the signal for HPV 13 (arrows) in the two white sponge nevus cases, corresponding to the H&E s of Fig. 1G and H/I, respectively.

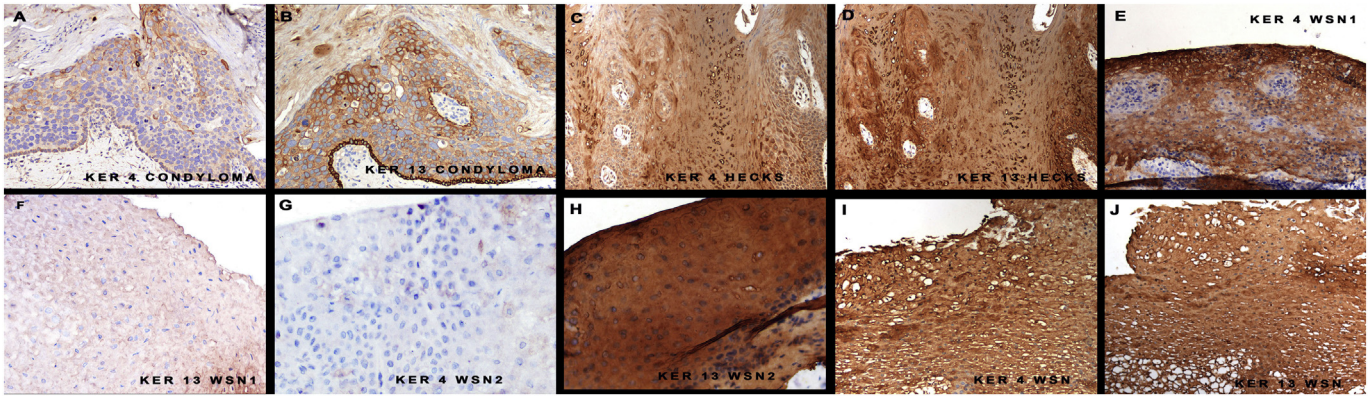

Fig. 3. Analyses for cytokeratins 4 and 13 in the oral condylomata, Heck's disease, and white sponge nevus. Panel A/B shows the strong signal for cytokeratins 4 and 13, respectively, in the oral condylomata. Panel C/D shows similar results for a Heck's disease case. Panels E/F and G/H show that the two cases of white sponge nevus negative for HPV showed either the loss of cytokeratin 4 or 13. Panel I/J show that the white sponge nevus cases with HPV 13 showed strong expression of both cytokeratins.

Table 1
Compilation of HPV DNA in situ data and immunohistochemistry for cytokeratins 4 and 13.

Category	HPV 6/11	HPV 13	Cytokeratin 4	Cytokeratin 13
Oral condylomata	6/6	0/6	6/6	6/6
Heck's disease	0/2	2/2	2/2	2/2
White sponge nevus case 1	0	0	**Positive**	Negative
White sponge nevus case 2	0	0	Negative	**Positive**
White sponge nevus case 3	0	Positive	**Positive**	**Positive**
White sponge nevus case 4	0	Positive	**Positive**	**Positive**

condylomata showed a strong signal for cytokeratin 4 and 13 (Fig. 3, panels A, B). Similarly, the HPV infected squamous cells in Heck's disease showed a strong signal for the two cytokeratins (Fig. 3, panels C, D). The two cases of white sponge nevus that were HPV DNA negative showed either a signal for cytokeratin 4 and none for cytokeratin 13 (Fig. 3, panels E, F) or the reverse pattern (Fig. 3, panels G, H). Finally, the two cases of diagnosed as white sponge nevus on clinical grounds that were HPV 13 positive each showed a strong signal in the squamous cells of the lesion for cytokeratin 4 and 13 (Fig. 3I, J). A summary of the clinical, HPV DNA, and cytokeratin 4/13 data for each of the cases is provided in Table 1.

3.4. Biomarker analyses

We had previously shown that HPV 6/11 condylomata of the vulva do not express the biomarkers that are typical of high grade HPV infection (cervical and vulvar intraepithelial neoplasia) that include p16, importin-β, exportin-5, and Mcl1 [24]. To address this question with Heck's disease, each tissue was tested for these proteins as well as Ki 67 by immunohistochemistry. Three case of VIN 2 served as positive controls.

Each of the three cases of VIN 2 was strongly positive for the panel of biomarkers p16, Ki 67, Mcl1, importin-β, and exportin-5 (data not shown). The biomarker Ki 67 showed variable expression in the condylomata, at times with a baseline signal involving only the basal cells of the lesion and other times with expression in cells more towards the apical aspect (Fig. 4, panel A). However, the classic high-grade HPV biomarker p16 was absent in each of the condylomata (Fig. 4, panel B) as well as each case of Heck's disease (Fig. 4, panel C) and white sponge nevus. Ki 67 showed a signal in only the basal cells of the Heck's disease and white sponge nevus cases (Fig. 4, panels D, E). None of the other biomarkers of high risk HPV disease, including importin-β, exportin-5 (Fig. 4, panel F) or Mcl1 were evident in any of the condylomata, Heck's disease or white sponge nevus cases.

Each lesion was also tested for the HPV L1 capsid protein using a consensus antibody (Biocare Medical, Pacheco CA) that can detect this viral protein in a wide range of papillomavirus infections though it has not been tested against HPV 13. Each of the six condylomata were positive for the L1 capsid protein (Fig. 1, panel B) but none of the Heck's disease or white sponge nevus showed a signal using the L1 capsid protein antibody (data not shown).

4. Discussion

The main focus of this study was to compare the histologic and molecular correlates of papillomatous lesions that occur in the oral cavity, especially in the lip region, and that also can occur in the skin. The paper focused on three such lesions that show overlap with the clinical presentation: oral condylomata, Heck's disease, and white sponge nevus. Although condylomata and Heck's disease are each due to an acute HPV infection, the former is sexually transmitted whereas Heck's disease is not and, thus, the clinical implications of the two diagnoses are very different. The main finding of this study was that a panel of tests including in situ hybridization for HPVs 6/11, 13, and immunohistochemistry testing for cytokeratins 4 and 13 could unequivocally separate the 12 cases examined in this study into one of the three categories: condyloma (HPV 6/11 positive), Heck's disease (HPV 13 positive), and white sponge nevus (cytokeratin 4 or 13 negative). Differentiating condyloma from white sponge nevus from Heck's disease can at times be done on clinical and/or histologic grounds, but, as shown in this study, may in fact require molecular testing to be definitive. It is important to stress that several cases diagnosed as white sponge nevus, which is often transmitted as an autosomal dominant disease, were indeed Heck's disease as manifested by the panel of testing. Another important point for the diagnostic surgical pathologist is that there was considerable overlap in the histologic features of the three diseases. Oral condylomata, Heck's disease, and white sponge nevus each showed marked acanathosis/papillomatosis, as well as para- and hyperkeratosis. However, the oral condylomata and Heck's disease each did show prominent perinuclear halos while the white sponge

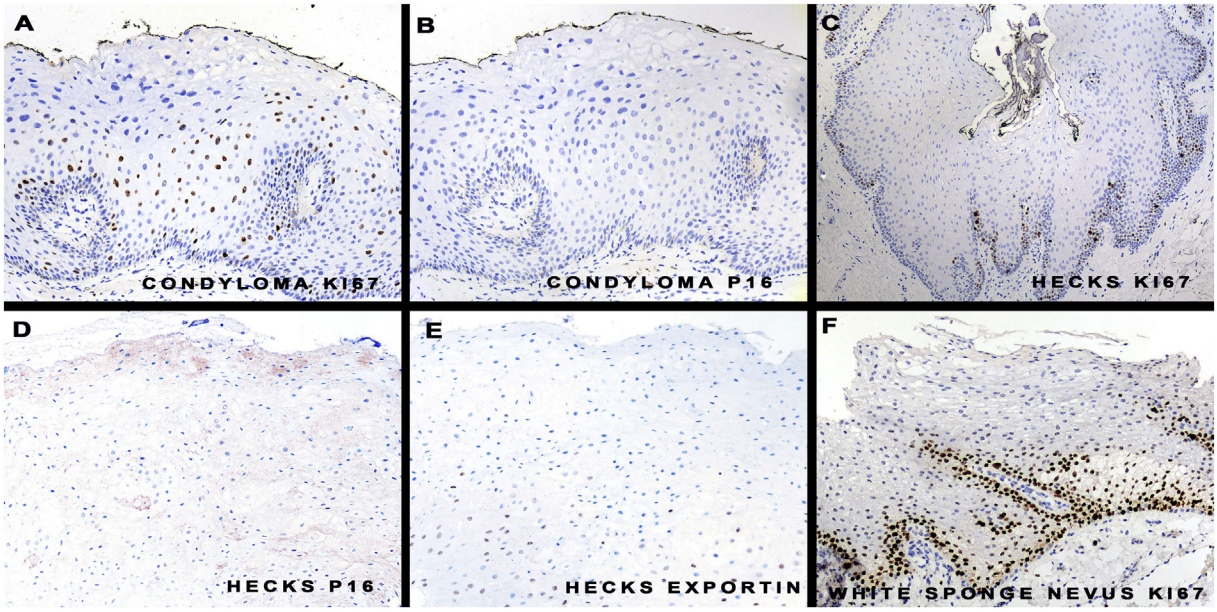

Fig. 4. Biomarker analyses in the oral condylomata, Heck's disease, and white sponge nevus. Panel A/B shows that Ki 67 at times showed expression in cells above the baseline in oral condylomata but that p16 was invariably negative. The Heck's disease cases showed a baseline Ki 67 signal (panel C) and no signal for the high risk HPV biomarkers p16 or exportin-5, respectively (panel D/E). The white sponge nevus cases also showed a baseline signal for Ki 67 (panel F).

nevus cases tended to show more degeneration of keratinocytes.

The first case reports of Heck's disease were published by Estrada in 1956 and 1960, followed by Soneira and Fonseca extensively documenting clinicopathological findings in 1964 [6,10]. Archard and Heck published their findings later in 1965, who used the term multifocal epithelial hyperplasia [6]. The clinical manifestation of Heck's disease is often multiple, asymptomatic, round, 5–10 mm diameter, sessile or exophytic papules or nodules in the oral mucosa in children and young adults [1,3]. A characteristic feature on physical exam is the disappearance of nodules when stretched and reappearance when released due to the confluence of small nodules into larger formations [6]. The disease course is one of spontaneous remission and recurrence, with a widely variable duration of this pattern from weeks to years [6].

White sponge nevus is an autosomal dominant disease that affects primarily the oral mucosa. The characteristic mutations in either cytokeratin 4 or 13 cause reduced desquamation of the keratinocytes towards the apical surface of the lesion. Thus, the lesions resemble leukoplasia, often on the inside of the mouth near the junction with the lips. However, unlike leukoplakia associated with tobacco chewing, there is no atypia and no known risk with cancer. As with Heck's disease, the lesions are usually evident in childhood, tend to be prominent, and tend to wax and wane over time. Surprisingly, the biopsies of white sponge nevus and Heck's disease analyzed in the present study were all from adult patients. This finding underscores that pathologists need to consider the diagnosis even in adults. In comparison, oral condylomata tend to occur in young, sexually active adults, although it is well documented that their appearance in pre-pubescent children can be an indicator of sexual abuse.

As indicated, HPV testing and typing is a straightforward way to differentiate oral condylomata from Heck's disease and white sponge nevus. PCR subtyping for HPVs 6/11, 13 and 32 is an option since standard assays only test for more common genital tract HPV types such as HPVs 6/11 and 16 [2]. However, given the high copy number of HPV 13 DNA in these lesions, in situ hybridization using a genomic HPV 13 and 32 probes should be definitive.

Few studies have investigated biomarkers in lesions with low-risk HPV types, such as those responsible for Heck's Disease, condyloma accuminatum, or verruca vulgaris. p53 has shown promise as a marker of low-risk disease due to overexpression in condyloma acuminatum [28] and decreased expression in the higher risk Bowen disease, [29] however results have been variable. Cyclin D1 is also being explored due to its correlation with low-risk HPV infection [28]. We recently documented that high risk HPV infections but not those due to HPVs 6/11 are associated with a panel of biomarkers including p16, Mcl1, importin-β, and exportin-5 [24]. In the present study we documented that HPV 13 in Heck's disease is likewise not associated with these biomarkers, underscoring that it is indeed a benign HPV type with no risk of malignant transformation. With respect to Ki 67, this study showed that oral condylomata, Heck's disease, and white sponge nevus all tended to show the same pattern of expression, with signal confined mostly to basal squamous cells of the lesion. This suggests that marked squamous cell proliferation is not a feature of any of these diseases, unlike high risk HPV infection where the Ki 67 proliferation index is invariably much increased [24].

The immunohistochemistry for cytokeratins 4 and 13 is a simple, standard test that can be performed in any diagnostic pathology laboratory; each antigen is easily exposed with antigen retrieval. In situ hybridization is a less resource intensive alternative to PCR for identifying low-risk HPV types. Although HPV DNA in situ hybridization generally carries a lower sensitivity than PCR, [30] the high viral copy number in Heck's Disease renders HPV DNA in situ hybridization uniquely useful. While HPV capsid L1 protein immunohistochemistry shows promise as a marker of low-grade proliferative lesions in the literature, the present study was not able to evaluate this due to the particular L1 antibody subtype used, due to insufficient homology with the L1 from HPV 13 [31,32].

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Declaration of competing interest

None.

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Disclosure

There is no duality of interests to declare.

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