Case Report

Oral Focal Epithelial Hyperplasia: Report of 3 Cases With Human Papillomavirus DNA Sequencing Analysis

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Abstract: *Focal epithelial hyperplasia (FEH), or Heck's disease, is a benign proliferative viral infection of the oral mucosa that is related to Human Papillomavirus (HPV), mainly subtypes 13 and 32. Although this condition is known to exist in numerous populations and ethnic groups, the reported cases among Caucasians are relatively rare. It presents as asymptomatic papules or nodules on the oral mucosa, gingiva, tongue, and lips. Histopathologically, it is characterized by parakeratosis, epithelial hyperplasia, focal acanthosis, fusion, and horizontal outgrowth of epithelial ridges and the cells named mitozoids. The purpose of this case report was to present 3 cases of focal epithelial hyperplasia in a pediatric age group. Histopathological and clinical features of cases are discussed and DNA sequencing analysis is reported in which HPV 13, HPV 32, and HPV 11 genomes are detected. (Pediatr Dent 2011;33:522-4)*
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Focal epithelial hyperplasia (**FEH**), also known as Heck's disease and/or "multifocal papilloma virus epithelial hyperplasia, is a benign proliferative viral infection of the oral mucosa. The cause of the disease is human papillomavirus (**HPV**); in most cases, the DNA of HPV 13 and, less frequently, HPV 32 has been identified.[1] Although this condition is known to exist in numerous populations and certain ethnic groups of younger ages, the reported cases among Caucasians and elderly people are relatively rare, and a family history and endemic occurrence are possible. Its clinical course is variable, although it usually persists for months or years; cases with spontaneous resolution have been described, as have others with prolonged persistence.[2,3]

Initially described in children, this condition is now known to affect patients in a wide age range. An equal gender distribution has been noted. FEH is characterized by the presence of multiple, circumscribed, sessile, soft, elevated papules or nodules. Their color ranges from pale to normal as compared to the adjacent mucosa. The lesions are asymptomatic.[2,3]

Histologically, it is characterized by acanthosis, parakeratosis, fusion, and elongation of epithelial ridges. One of the most prominent features of FEH, the so-called FEH cells or mitozoids, can be found at various levels of the epithelium, showing ballooning with abnormal nuclear chromatin pattern. Dyskeratosis and epithelial atypia have never been described in FEH.[1,2]

The purpose of this study was to determine human papillomavirus subtypes 13, 11, 32, and 6 in 3 focal epithelial hyperplasia cases via nested polymerase chain reaction (**NPCR**).

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The clinical properties of the cases are summarized in Table 1. The patients' ages were 8, 16, and 17; 2 were female and 1 was male. Lesions were multiple with nodular or papullonodular appearances in all cases and were located mainly on the inner aspect of the lips. The size of the lesions were between 0.4 to 1 cm in diameter, and their color ranged from pale to red, or was similar to the healthy adjacent mucosa (Figure 1). Duration periods of the cases are given in Table 1. All lesions were asymptomatic. One out of 3 cases presented with a family history. An 8-year-old female patient with multiple extensive lesions was informed that her sister and father also had similar lesions, which were resolved spontaneously.

The clinical diagnoses of the presented cases were papilloma, FEH, and condyloma acuminatum. For the definitive diagnosis, incisional biopsies were performed.

The histopathological examination revealed acanthosis, hyperkeratosis, proliferation and fusion of epithelial ridges of the squamous epithelium (Figure 2). Enlarged ballooning cells with abnormal chromatin patterns were seen in the spinous layer. Cells beneath the superficial layer showed pyknotic nuclei with surrounding clear halo and nuclear fragmentation (Figure 3).

Immunohistochemistry was used to detect HPV genus specific antigen by avidin biotin peroxidase complex method. An HPV positive papilloma case was used as a positive control, and the same tissue incubated with PBS was used as a negative control. Nuclear red staining of the cells at the squamous epithelium were accepted as positive for HPV. We didn't, however, detect any positive staining.

The presence of HPV DNA was identified by NPCR and sequencing analysis. HPV genomic DNA of samples was detected with a highly sensitive NPCR assay. The degenerate primers MY09/MY11 (outer) and GP5+/GP6+ (inner) were used to

Table 1. CLINICAL FEATURES AND HUMAN PAPILLOMAVIRUS (HPV) STATUS OF FOCAL EPITHELIAL HYPERPLASIA CASES

Case	Gender	Age	Localization	Clinical appearance	Diameter size (cm)	Duration (mos)	HPV (IHC)*	HPV type
1	Female	17	Upper and lower lip mucosa	Papillary-nodular	0.4-1	6	-	13
2	Male	16	Upper lip mucosa	Papillary-nodular	0.5	9	-	32
3	Female	8	Upper and lower lip mucosa, tongue	Papillary-nodular	0.8	14	-	13/11

* IHC= Immunohistochemistry.

detect a general HPV positivity which direct the amplification of 450 bp and 150 bp, respectively.[4,5] HPV genotyping was done by direct DNA sequencing analysis (Open Gene System, Visible Genetics Inc., Toronto, CA). Sequence data was interpreted using the Gene Objects 3.1 software (Visible Genetics, Canada) for HPV 13, 11, 32, and 6 sequences from the Gen Bank Database (National Center for Biotechnology Information, Bethesda, Md). NPCR analysis showed that all cases' samples were HPV DNA positive (Figure 4). In 1 sample case, coinfection by HPV 11 and 13 was detected (Table 1).

All lesions were treated by cryosurgery. Liquid nitrogen (-196°C) was applied via spray technique (Brymill, Cryogenic Systems LD, UK). Two consecutive 15-day treatments were required to eliminate the lesions. After a 24-month follow-up period, a recurrence of the lesions was not observed.

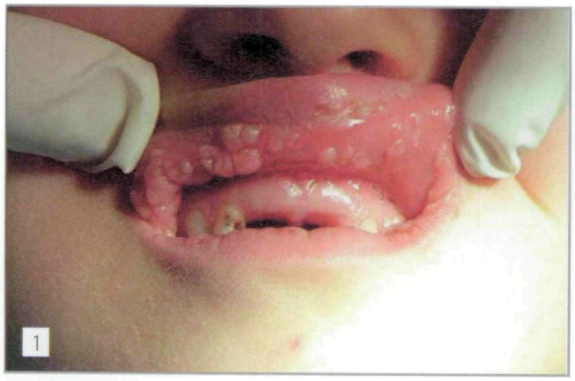
Discussion

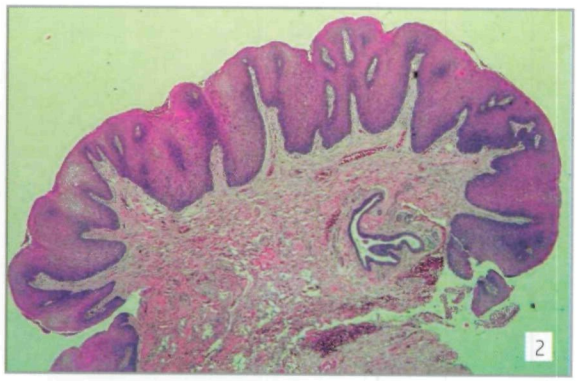
FEH is a rare disease of the mucous membranes, first described by Archand et al. in 1965.[6] It follows a benign course that normally appears in childhood and adolescence, mainly between 3 and 18-years-old, and is clearly associated with HPV serotypes 13 and 32.[1,2]

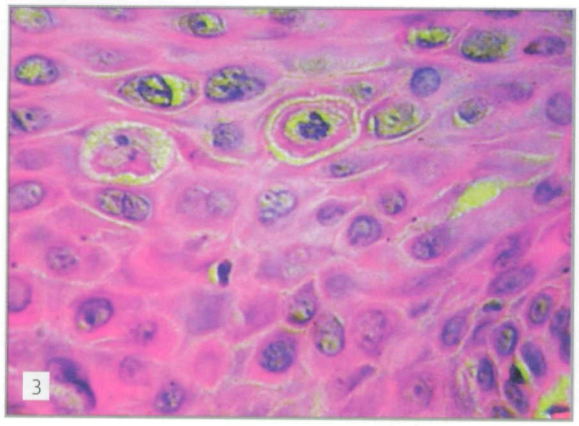
This entity has distinctive clinical and histological characteristics. Clinically, it is characterized by multiple flesh-colored papules of 3 to 10-mm that tend toward confluence and produce a cobblestone appearance in the mucosa. The histological characteristics include epithelial hyperplasia, elongation, and anastomosis of the interpapillary ridges, parakeratosis, focal acanthosis, focal koilocytosis, and the presence of structures resembling mitotic figures in superficial keratinocytes.[1-3]

Both clinical and histopathological features of the cases were consistent with FEH. Garlick and Taichman[7] demonstrated identical types of HPV in affected siblings and first-degree relatives of affected patients, supporting both viral etiology and genetic predisposition. Recent studies demonstrated the presence of HPV types 13 and 32 in most FEH cases.[8-10]

We could not detect any HPV positivity by immunohistochemistry. It reveals that immunohistochemistry may not be a reliable method for detection of viruses, which was reported in







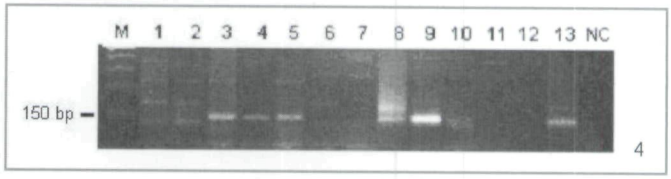


Figure 1. Multiple sessile, soft, elevated pink papules in the labial mucosa.

Figure 2. Acanthotic squamouse epithelium with horizontal outgrowth of epithelial ridges (hematoxylin and eosin 40X).

Figure 3. Ballooning cells with abnormal nuclear chromatin pattern (hematoxylin and eosin 4X).

Figure 4. Representative gel of human papillomavirus (HPV) polymerase chain reaction. M=marker; lines 3, 4, and 5=focal epithelial hyperplasia cases; N=negative control (water). Lanes 1-2 and 6-13 were papilloma cases detected for HPV for another unpublished study.

previous studies. Several techniques have been used to detect the presence of HPV in tissues: immunohistochemistry; southern-blot analysis, previously considered to be the most sensitive assay; or in situ hybridization analysis, which can be performed on parafin-embedded sections. Those techniques have the disadvantage of requiring a sizable quantity of nucleic acids and take several days to perform. As DNA amplification methods permit more sensitive detection of viral DNA, PCR is a far more sensitive and specific molecular method to identify any viral genome in the tissue.[11] The presence of HPV was also studied by means of nested PCR, and DNA sequencing analysis was performed to determine the HPV subtypes.

In this study, we detected HPV 13 and HPV 32 in 2 cases; 1 case showed positivity for both HPV 13 and HPV 11. The 8-year-old patient with family history showed positivity for both HPV 13 and HPV 11 and had more extensive lesions of FEH, which may be due to coinfection.

To the best of our knowledge, coinfection with 2 or more types of HPV has not been reported in any FEH cases yet. In some studies, a reaction with other subtypes, in particular HPV 6 and HPV 11, has been reported.[12,13] As the HPV-DNA typing of our lesions were performed by DNA sequencing analysis, we may suggest that there was a coinfection of HPV13 and HPV 11 more than a cross reaction of the pathogens.

In the present case reports, we detected HPV by NPCR using the degenerate primers **MY09/MY11 (outer) and GP5+/GP6+ (inner) primers**[4,5] and performed sequencing for subtyping of HPV. In previous studies, GP5+/GP6+ primers and sequencing analyses of the amplified fragments were reported as a reliable method for HPV identification.[5,10]

HPV 13 and HPV 32 are the implicated viruses in over 90% of FEH cases,[14] but population-based studies showed that different HPV subtypes seem to be related in pathogenesis of FEH in different populations or ethnic groups,[4,10] which requires further assessment in the future.

Although subtypes of HPV are documented as etiologic agents, factors that determine disease susceptibility are unclear, but genetics, and having the human lymphocytic antigen-DR4 allele in particular, are thought to play a major role in vulnerability to HPV 13 or HPV 32 viruses.[14] Low socioeconomic status, malnutrition, and poor hygiene are also considered to be important factors for the development of FEH. These factors lead to chronic immunosufficiency, which facilitates the existence and easy transfer of the infection.[15] In the present cases, all the patients were of low socioeconomic status.

The differential diagnosis should include florid oral papillomatosis, verrucae vulgaris, papilloma, and condylomas, particularly in young patients, to avoid inaccurate sexual or abuse-related implications.

Overall, FEH is a self-limiting disease which tends to regress spontaneously; however, some cases may progress or persist for several years. Those persistent and extensive FEH lesions must be detected for the possible co infection. Cryosurgery seems to be a good treatment modality for extensive lesions.

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