Koilocytes in Oral Pathologies

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ABSTRACT

Introduction: Oral HPV infections affect 1 to 50% of the general population. Naturally, about 90% of HPV is eliminated by the immune system, but the ones that persist may result in serious diseases. Human papillomavirus is the main cause of cancer at various body sites, such as cervix, uterus, oropharynx, head and neck. The prevalence of oral HPV infections in India ranges from 15 to 16%. About 80% of HPV infections are present with koilocytosis as an important morphological marker.

Aim: This review focuses on the importance of koilocytes and its early detection to alert malignant risk for facilitating human papillomavirus (HPV)-targeted therapeutic strategies.

Results: Research in the past has primarily focused on cervical cancer, as >99% of them harbor HPV. It has been observed that the incidence of HPV-associated cancers may be minimized by effective preventive and targeted therapeutic modalities. Although oral HPV infections have been minimally researched lesions such as verruca vulgaris, squamous papilloma, focal epithelial hyperplasia and condyloma acuminatum are significantly prevalent in India. The characteristic cell observed in all these HPV-associated lesions is koilocytes as demonstrated using immunohistochemistry (IHC) with p16 antibody. Although advanced techniques, such as polymerase chain reaction (PCR), deoxyribonucleic acid (DNA) breakage detection fluorescence in situ hybridization (DBD-FISH), and comet assay may confirm their presence, they are cumbersome and not economical.

Conclusion: Detection of malignancy-prone oral HPV infections by the demonstration of koilocytes showing positivity to p16 IHC stain has shown promising avenues in the field of research for the prediction of diagnosis and prognosis of oral squamous cell carcinomas (OSCC).

Clinical significance: Koilocytic cells are pathognomonic of HPV infection. Identification of koilocytes in histopathologic sections alerts the pathologist to suspect any underlying HPV infection and directs the surgeon for appropriate treatment. Human papillomavirus infection affects the function or host gene expression that is marked by cellular changes, and unmasking of such changes has an important role in the identification and follow-up of such patients.

Keywords: Human papillomavirus, Human papillomavirus-associated oral lesions, Koilocytes.


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INTRODUCTION

Human papillomavirus belongs to the family of viruses, the Papovaviridae.1 These are small, epitheliotropic double-stranded DNA viruses with more than 120 identified genotypes in human.1,2 There are specific HPV types responsible for the causation of cancerous and non-cancerous tumors.3,4 Human papillomavirus prevalence ranges from 22 to 60% in the normal mucosa that includes subclinical and/or latent infections of the oral cavity.3,4

Oral cancers are found to be commonly associated with HPV infection with an overall frequency of 74%, while 41% showed multiple HPV infections. About 10% of men in the age range of 14 to 69 years have an oral HPV infection compared with 3.6% of women.3,4

A higher percentage (more than 40%) is seen in HPV cancers at the base of tongue and of palatine tonsils.3,5 Recent studies have demonstrated that the association between HPV and OSCC is stronger in young males than in females. There is a wide geographical variation in the prevalence of HPV among Indians with 27% of oral cancer from North India being positive for HPV 16, whereas 25 to 31% positivity in western India.1,2,6

KOILOCYTES

These are squamous epithelial cells that have endured a number of structural changes due to HPV infection. The infected cells have an eccentric, hyperchromatic, and enlarged nucleus that is displaced by a large perinuclear vacuole showing irregular contour and appearing crenated, “raising-like” or “spoon-like” in shape.7,8 Hence, the name “koilocyte” is derived from Greek, in which koilos means “hollow” and “cyte” means cell (Fig. 1).5,7,8

The role of cytoplasmic vacuolization in viral replication is unclear. However, it could contribute to the fragility of keratinocytes and release of viral-laden nuclei from HPV lesions.1,5,8 There is a movement of papillomavirus to the superficial layers of the epithelium that is facilitated by disturbing keratin integrity and assembly.
of cornified layer. Collectively these changes are called cytopathic effect. Some cells may display binucleation with nuclear atypia and are termed as “koilocytic dysplasia”.5,7-9

Although koilocytosis denotes an important morphological marker for HPV infection, there are instances wherein artifacts caused due to fixation, processing, or surgical errors mimic koilocyte-like appearance.

**Pathogenesis**

The actual mechanism by which HPV enters the cell is still unclear. It is accepted that the entry of the virus into the epithelial cell and delivery of DNA into the nucleus is through HPV capsid proteins. Infection occurs when the virus penetrates micro injuries and enters the new host. There are three factors that control the development of incubation phase into active expression: Cell permeability, virus type, and the host immune status.5,9

The sequential steps involved in the pathogenesis of HPV infection have been depicted in Flow Chart 1.

**HUMAN PAPILLOMAVIRUS TYPES**

Currently, around 100 types of HPVs have been identified, but only a few (e.g., HPV 16, 18, 31, and 33) are known to be associated with malignant risk. Human papillomavirus types have been broadly categorized into high- and low-risk types3,4 as depicted in Table 1.

**IMPORTANCE OF KOILOCYTES**

Koilocytosis is the most common cytomorphologic change and is considered by pathologists to be a major histopathologic feature for the determination of HPV
**Table 1: Human papillomavirus types and their significance**

<table>
<thead>
<tr>
<th>Relative risk</th>
<th>Positive HPV types</th>
<th>Significance</th>
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<tbody>
<tr>
<td>High risk</td>
<td>Most common: 16 and 18</td>
<td>Associated with malignancy</td>
</tr>
<tr>
<td></td>
<td>Others: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68</td>
<td>Benign neoplasms</td>
</tr>
<tr>
<td>Low risk</td>
<td>6, 11, 40, 42, 43, 44, 53, 54, 61, 72, 73, 81</td>
<td></td>
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infection. In cervical neoplasias, koilocytosis has been classified as (Bethesda system, 2001).\(^9\)\(^{-11}\)

- **Low squamous intraepithelial lesion:** mild koilocytosis with mild dysplasia/cervical intraepithelial neoplasia (CIN1).
- **High squamous intraepithelial lesion:** marked koilocytosis with moderate and severe dysplasias, carcinoma in situ/CIN 2 and 3.

Oral koilocytic dysplasia is a distinct entity that shows HPV in light microscopy with 80% accuracy.\(^8\) However, the clinical connotation and probability for malignant risk needs to be investigated further. Positive cases of koilocytic dysplasia contain HPV types 31, 33, 51, 16, and 18.\(^2\)\(^,\)\(^11\) In HPV infection, koilocytes are a significant microscopic finding in association with features of conventional epithelial dysplasia.\(^11\)

A study undertaken by Chaturvedi et al in the United States evaluated changes in patient characteristics, incidence and survival in OSCC sites between HPV-related and HPV-unrelated areas. They concluded that the prevalence of HPV-related OSCCs was identified at an earlier age group compared with HPV-unrelated OSCCs. Similar results were observed with 2 years survival following radiation therapy.\(^12\)

**HUMAN PAPILLOMAVIRUS-ASSOCIATED ORAL LESIONS**

The prevalence of oral HPV infections in India ranges from 15 to 16%.\(^2\)\(^,\)\(^13\) Koilocytes are generally found in association with HPV oral lesions that include verruca vulgaris, squamous papilloma, focal epithelial hyperplasia, and condyloma acuminatum.

Squamous papilloma, a benign neoplasm of squamous cells, affects predominantly the tongue, buccal mucosa, and lips. Histopathologically, the intermediate layers of epithelium demonstrate koilocytes which predominantly show positivity for HPV 6 and HPV 7.\(^1\)\(^,\)\(^2\)\(^,\)\(^13\) Verruca vulgaris is a common benign skin lesion but can be present in oral cavity also. The preponderance of mucosal warts is seen in children and associated with HPV 2 and 4 types. The variants of warts could be flat warts associated with HPV types 3 and 10 and butcher's warts with HPV 7. Numerous koilocytes (89%) are often seen in the superficial spinous layer.\(^13\) Focal epithelial hyperplasia/Heck's disease is a rare benign oral lesion caused by HPV 13 or 32 with a site specificity of keratinized and nonkeratinized surfaces respectively, in the superficial layers.\(^14\)

Oral condyloma acuminatum that commonly affects buccal mucosa, floor of mouth, lingual frenum, and hard palate is induced by HPV types 6 and 11. Mild to moderate numbers of koilocytes are appreciated in superficial layer.\(^15\)

Oral lichen planus is a chronic immune-mediated disease, presenting with bilaterally symmetrical lesions that affect the gingiva, lip mucosa, and dorsum of tongue. The HPV types 11 and 16 are commonly associated with the lesion with koilocytes predominantly found in stratum granulosum.\(^13\) Oral leukoplakia is a precancerous lesion with a malignant transformation rate of 16 to 62%. The lesion is most commonly seen on the buccal mucosa, tongue, and palate and may be caused by HPV types 6, 11, and 16 and may rarely display koilocytes.\(^2\)\(^,\)\(^16\)

Verrucous carcinoma, a variant of squamous cell carcinoma, occurring on lips, oropharynx, and larynx, is usually associated with HPV 6, 11, 16, and 18. The presence of koilocytes is seen in the intermediate layer of the epithelium.\(^5\)\(^,\)\(^6\) Oral squamous cell carcinoma is usually associated with HPV 16, 11, and 27 with a frequency of 21 to 60%. Allocation of koilocytes is usually in the form of scattered foci throughout the epithelium which suggests nonclonal origin of tumor nature.\(^6\)

**DEMONSTRATION OF KOIELCYES IN RELATION TO A SUSPECTED CASE OF VERRUCOUS CARCINOMA USING IHC**

**Routine and Special Stains**

A clinically suspected case of carcinoma that histopathologically revealed numerous koilocytic cells in the superficial epithelium was employed for staining with IHC (p16). Hematoxylin and eosin (H&E)-stained section showed numerous cells in the stratum spinosum and granulosum layers that had a perinuclear halo- and crescent-shaped nucleus, suggestive of koilocytes (Fig. 2A). Immunohistochemistry using p16 antibody was performed to confirm the presence of high-risk HPV infection. Immunohistochemistry showed brown-colored staining in the cytoplasm, nucleus, and nuclear membrane suggestive of strong positivity for p16 as shown in Fig. 2B. As the high-risk HPV marker was strongly positive, the present case was suspected for malignant risk.

Few of the studies favoring our results are as follows: Patil et al\(^17\) evaluated the expression of HPV in OSCC and correlated its association with histological grades using p16. The researchers found strong association between HPV infection and OSCC. The diffuse pattern of p16 positivity noted in poorly differentiated OSCC cases depicted the increased viral overload, which might have an influence on its aggressive behavior.
An advanced technique that permits cell-to-cell detection and DNA breakage quantification is DBD-FISH that utilizes whole genome or specific DNA areas. The purpose of this method is to recognize HPV infection through changes in chromatin due to increase in the number of alkaline labile sites. These findings may be crucial for the investigation of mechanisms involved in viral tumorigenesis in humans and have been justified by the study done by Cortés-Gutiérrez et al.\textsuperscript{21}

Comet assay or single cell gel electrophoresis is a rapid and highly sensitive fluorescent microscopy–based method to examine DNA damage and repair at the level of individual cells. This technique is usually applied for the detection of protein depletion of HPV. A study by Cortés-Gutiérrez et al\textsuperscript{22} was performed to determine the relation of DNA single (ssb) and double (dsb) strand breaks in women with and without cervical neoplasia using comet assays. A significant increase in global DNA damage (ssb + dsb) and dsb was observed in patients with high-grade squamous intraepithelial lesion (SIL; 48.90 ± 12.87 and 23.50 ± 13.91 respectively), patients with low-grade SIL (33.60 ± 14.96 and 11.20 ± 5.71 respectively), and controls (21.70 ± 11.87 and 5.30 ± 5.38 respectively), and they concluded that real genomic instability favors women with cervical neoplasia, having higher proportion of DNA dsb.

Nucleic acid hybridization technique involves Southern blot, DB, and reverse dot hybridization methods. It uses radiolabeled nucleic acid hybridization assays to detect HPV infection. Sensitivity of these techniques is relatively low compared with PCR. Hence, currently, these techniques are rarely used.\textsuperscript{23}

Hybrid capture (HC) technology ascertains nucleic acid targets directly using signal amplification to provide sensitivity comparable to target amplification methods (PCR). It is helpful in the identification of high-risk HPV
types. Chaudhary AK et al conducted a study to compare the efficacy of HC-II assay and PCR for the detection of specific HPV type (HPV 16 E6) in oral submucous fibrosis (OSMF) and OSCC. They found high-risk HPV 16 E6 DNA positivity in nearly 27.4 and 31.4% in OSMF and OSCC respectively, and concluded that HC-II assay had better sensitivity in cases of OSCC.

CONCLUSION

Although routine histopathology demonstrates koilocytic cells, IHC and advanced techniques, such as PCR, DBD-FISH, and comet assay are necessary to confirm these cells. Most of the HPV research has primarily focused on cervical cancer, as >99% of them harbor HPV. In the future, the development of preventive and therapeutic strategies may reduce the incidence and mortality of HPV-associated cancers. Oral HPV infections have been minimally explored in comparison with cervical HPV infections. However, in recent years, evidence of HPV infection is emerging as an important marker for predicting the prognosis of head and neck squamous cell carcinoma. Furthermore, its role in habit-associated Indian oral cancers is questionable. Hence, there is a necessity to study oral HPV infections more extensively, so that cancer prevention can be effectively put into practice using HPV vaccines.

REFERENCES