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Original Research

Study of Prevalence of Koilocytes in Oral Squamous Cell Carcinoma

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ABSTRACT

Ever since Dr. Harald zur Hausen won the noble prize in medicine in 2008 for discovering human papilloma virus (HPV) as the etiological factor for cervical cancer, the research has been robust in this field. More recent data from case–control studies and systematic review indicate that HPV is an independent risk factor for oral and oropharyngeal carcinomas. Oral squamous cell carcinoma (OSCC) represents 90% of all malignant tumours that affect the oral cavity. The most known viral cytopathic effect is koilocytosis, considered to be a major characteristic of HPV infection. Koilocytes are epithelial cells characterised by perinuclear haloes surrounding condensed nuclei and is accepted as pathognomonic (characteristic of a particular disease) of HPV infection. **Aim:** The aim of this study was to verify the prevalence of koilocytes in OSCCs. **Methodology:** A retrospective analysis was conducted on 60 paraffin-embedded tissue specimens that were obtained from patients with a histopathological diagnosis of OSCC. Haematoxylin and eosin-stained slides were submitted to examine under light microscopy, specifically for the determination of the presence of koilocytes and were analysed. **Conclusion:** The results suggest that the presence of koilocytes is reliable for the detection of HPV presence in routine histopathology in OSCCs.

KEYWORDS: Oral squamous cell carcinoma, Oral cancer, HPV, DNA Virus, Oral carcinogenesis

INTRODUCTION

In India, oral cancer constitute around 9.8% of total cancer cases and ranks first among the all cancer cases in males and third most common among females. Age standardised incidence rate varies between 7 and 17/ 1000,000 person/years. The incidence rate is higher than the rate of 3 to 4/1000,000/year found in the western countries [1–3]. Oral squamous cell carcinomas (OSCCs) are characterised by multiphasic and multifactorial etiopathogenesis. Tobacco and alcohol are the most common risk factors for oral malignancy. Other factors, including DNA viruses, especially human papilloma virus (HPV) have been documented to play a role in the initiation or development of these lesions [4].

HPV is epitheliotropic, and it may be present in a latent form, a subclinical form or in a form which in association with other ill-defined factors can induce benign or malignant epithelial neoplasms. Persistent high-risk HPV infection in a subset of subjects with HPV-cytopositive oropharyngeal epithelium induces genomic instability in these cells, making them susceptible to additional genetic alterations leading to cell transformation and to subsequent development of squamous cell carcinoma (SCC) [5]. Oral HPV infection is acquired primarily by sexual transmission, or less frequently by non-sexual direct transmission, by mother to child transmission or by autoinoculation. Persons who practise oral-genital sex, those who have had a number of sexual partners and those who are immunocompromised are at greater risk of acquiring oral HPV

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infection. Many of the low-risk genotypes and uncommonly high-risk types have been found in the HPV-associated benign oral lesions, oral squamous cell papilloma, verruca vulgaris (common wart), condyloma acuminatum and focal epithelial hyperplasia (heck disease), collectively termed oral warts. The four types of oral warts share the characteristics of being exophytic, sessile or pedunculated, or of having filiform or 'cauliflower'-like surface. The lesions, can be single, multiple or clustered, are usually painless and chronic and occasionally regress spontaneously [6]. High-risk HPV genotypes, in particular HPV-16 and -18, are prevalent in potentially malignant oral epithelial lesions, and in OSCC; in particular, HPV-16 have been implicated in the development of OSCC [7]. The importance of HPV infection in oral carcinogenesis is supported by the ability of high risk HPV to immortalise oral keratinocytes *in vitro* [8].

Koilocytosis first described by Leopoldo Koss *et al*. in 1956 is a long-recognised, pathognomonic feature of HPV infection. Koilocytes are commonly present in cervical intraepithelial neoplasia. They are epithelial cells characterised by perinuclear haloes (cytoplasmic vacuolation) surrounding condensed nuclei. Koilocytosis is accepted as pathognomonic (characteristic of a particular disease) of HPV infection (Reid *et al.*, 1982) [9–11].

Histopathological analysis under light microscopy is the most commonly used method for oral pathology diagnosis, and it is a useful method for the observation of viral particles when molecular biology methods are not available. To predict the presence of HPV infection, a retrospective analysis was conducted on 60 paraffin-embedded tissue specimens that were obtained from patients with a histopathological diagnosis of OSCC.

MATERIALS AND METHOD

This retrospective histological study was conducted on tissue sections obtained from the biopsy tissue specimens retrieved from Department of Oral Pathology and Microbiology, V.S. Dental College, Bangalore. The specimens were retrieved from the archives of the Department of Oral Pathology after getting the approval by the Institutional Ethics Committee. The samples were selected from exophytic verrucous or papillary OSCCs confirmed by histopathological diagnosis (Figures 1–3). No cases of verrucous carcinoma or other histological variants of SCCs were included in this study. For this study, 4-µm-thick sections obtained from the paraffin-embedded SCC samples were stained with haematoxylin and eosin. Analysis was carried out with emphasis on the grading and cellular details especially for the koilocytic changes and we based simplified histological definitions of koilocytosis on those established by Reid *et al*. (1982) as follows:

1. Koilocytic cytoplasmic vacuolisation: perinuclear haloes surrounding cell nuclei.
2. Koilocytic nuclear change: pyknosis – nuclear material that may be irregular in size, shape and staining properties. The nucleus is frequently acentric. Binucleation may be apparent. It is now known that 'binucleation' involves multilobules caused by HPV-influenced activity at the G2 cell cycle checkpoint (Cho *et al.*, 2005). In simple terms, large cells with perinuclear clearing take up a majority of the cells with associated nuclear atypia [10].

In this study, the presence of koilocytes in tissue was considered significant if more than five koilocytes per 10 high power fields (HPF) were seen in both neoplastic and non neoplastic areas (just surrounding the neoplastic area). The identification of koilocytes was determined by the presence of a halo or vacuole in the cell cytoplasm surrounding the nucleus (Figure 4).

RESULTS

Concomitantly, the demographic data from the requisition records of the 60 exophytic lesions of OSCCs were analysed. Forty-five percent of the cases (27/60) presented koilocytosis, and were identified as

positive cases of koilocytosis (K+) as shown in Table 1.

Table 1: Prevalence of koilocyte in OSSC cases

Koilocyte	Number of Cases
Positive	27/60
Negative	33/60

The patients' records showed 13 patients were male and 14 patient were female, ages ranging from 33 to 82 years (mean age = 48.9) in the K+ patients. Although, smokeless tobacco abusers were high in number (11) in this subgroup, most of the tumours were associated with both tobacco plus alcohol habit (23). Three patients did not have any history of habits in their records. The tumour location varied. In the positive K+ cases, gingivobuccal sulcus was the most prevalent site with 11 cases, and the second most prevalent site was buccal mucosa with 8 cases, 4 cases involved the hard palate, 2 cases of tongue and 1 case was involving the floor of the mouth.

Koilocytosis was found in 5–7 per HPF and was present in the 21 cases of well-differentiated carcinomas, in 11 cases of moderately differentiated SCCs and in 2 cases of poorly differentiated SCCs, which is tabulated in Table 2.

Table 2: Koilocyte positivity in different grades of OSSC

Well-differentiated SCC	14/31
Moderately differentiated SCC	11/24
Poorly differentiated SCC	2/5

The degree of koilocytosis was assessed separately in each section, and it was graded as percentile scores for the nuclear morphology of the koilocytes as shown in Table 3 and a score for the extent of vacuolation within the koilocytes as shown in Table 4 in percentages.

The shapes of the nuclei within koilocytes were also very variable, with rounded, irregular and sickle shaped nuclei being seen. The nuclei were either in a peripheral or central position.

In well-differentiated carcinomas, koilocytes showed changes in the cell with small dark nuclei and perinuclear clearing along with abundant cytoplasm (Figure 5). Pyknosis (intensely stained and irregularly shaped nuclei) was identified in a majority of cases but not in all of the koilocytes. Focal areas showed keratin formation surrounded by koilocytes exhibiting clear cytoplasm and dark stained nucleus (Figure 6).

In moderately differentiated and poorly differentiated carcinomas, altered epithelial cell with vesiculated nuclei and pale abundant cytoplasm were seen (Figure 7). Binucleation was seen in few cases (Figure 8).

Table 3: Nuclear morphology of koilocytes

	Description	%
Size	Larger than normal	23
Shape	Bizarre-angulated, to oval twisted	19
Staining	Dense, homogeneous, appearance often pyknotic	58

Table 4: Extent of vacuolation in koilocytes

	Description	%
Grade 1	Slight clearing to form a clear rim round the nucleus	32
Grade 2	Perinuclear clearing extending to half of cytoplasm	47
Grade 3	Extensive clearing, leaving only a thin rim of cytoplasm around cell periphery	21

DISCUSSION

HPVs are small double-stranded, circular DNA viruses that can infect epithelial cells. In squamous epithelium, the productive cycle of HPV is intimately linked with differentiation factors expressed within various layers of the host epithelial cell as koilocytes [11]. These changes predominantly affect superficial and intermediate cells and provide the most reliable evidence of HPV infection [12].

Koss *et al*. coined the term koilocyte for the large cells with relatively small but irregular and hyperchromatic

nuclei surrounded by clear and transparent cytoplasm. As the nucleus seem to be suspended in an empty space for descriptive purposes, they coined the term koilocytic atypia from the Greek word 'Koilos' meaning hollow or cavity as its designation. These koilocytes are squamous epithelial cells that contain an acentric, hyperchromatic nucleus that is displaced by a large perinuclear vacuole. However, the genesis of the cytoplasmic vacuole has remained unclear, particularly because both HPV DNA replication and virion assembly occur exclusively in the nucleus. In clinical biopsies, koilocytosis is observed in both low- and high-risk HPV infections; koilocytosis is promoted by the E6 oncoprotein, which is known to inhibit apoptosis [13,14]. Cytoplasmic vacuolisation could contribute to keratinocyte fragility and the release of viral-laden nuclei from HPV lesions [15].

From a biological point of view, HPV is a DNA oncovirus and is epitheliotropic. There are over 120 different HPV subtypes, including the low-risk types such as HPV 6 and HPV 11, responsible for benign proliferation of epithelium, and the high-risk oncogenic types HPV 16 and HPV 18 which are both well-established initiators of over 90% of cervical cancers, 70% of anogenital cancers, 5% of non-oropharyngeal SCC and 20–72% of (OPSCC) oropharyngeal SCC. The oncogenic nature of high risk HPVs is because of the immortalising and transforming properties of HPV oncoproteins E6 and E7, which target the p53 and pRB (**retinoblastoma protein**) tumour suppressor pathways, respectively, rendering infected cells susceptible to mutations and cancer formation [16].

The biology of HPV-positive cancer is typified by p53 degradation, retinoblastoma protein (rB) down-regulation and p16 up-regulation. By contrast, tobacco-related oropharyngeal cancer is characterised by p53 mutations, down-regulation of p16 and rB up-regulation [16].

A possible involvement of HPV in the development of precancerous lesions and oral carcinoma was first proposed by Syrjänen *et al.* (1983); based on light microscopy examination, they observed cytopathic HPV alterations (koilocytosis) in 35% of the OSCC biopsies that were identical to those found in precancerous lesions and uterine cervix carcinoma [17]. In a study based on HPV-related histopathological aspects, such as koilocytes, dyskeratosis, papillomatosis, hyperkeratosis, acanthosis and parakeratosis, koilocytes was considered as a pathognomonic sign of HPV-associated oral lesions [18].

In a recent study, there was statistically significant differences for koilocytes between HPV (16,18) positive and negative cases, which indicated that koilocytosis can be reliably used as markers for the diagnosis of HPV in OSCC [19].

Several studies have shown that patients with HPV-positive cancer have a significantly improved overall and disease-free survival compared with patients with HPV negative cancer patients.

Why does HPV positive oropharyngeal cancer have a better prognosis?

1. HPV-positive tumours may harbour fewer or different genetic alterations, which can be associated with better response to therapy.
2. HPV-positive tumours have higher radio sensitivity, probably due to intact apoptotic response to radiation.
3. The absence of field cancerization in HPV-positive tumours.
4. Immunologic response may play a role in the improved response to radio- and chemotherapy in HPV positive tumours (due to the stimulation of immune response directed to viral specific tumour antigens).
5. Younger age, good performance status, fewer comorbidities of HPV-positive oropharyngeal cancer patients may also contribute to improved survival [20].

In our study, the prevalence of HPV infection was around 45%, and the overall prevalence of HPV in OSCC in India has been reported as ranging from 20%

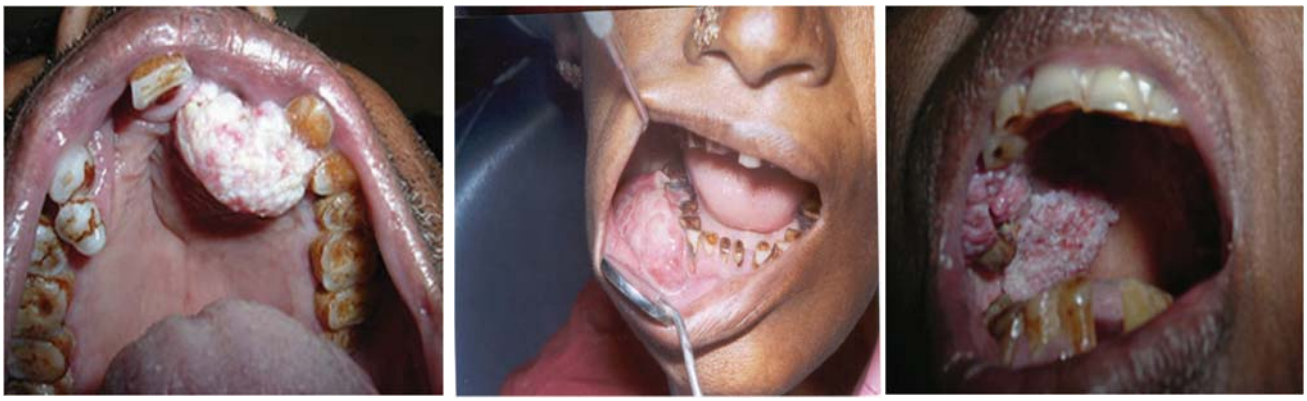

Figure 1,2,3: Figure clinical photomicrograph of oral cavity showing growth in right mandibular vestibule, maxillary anterior region, and palatal region respectively

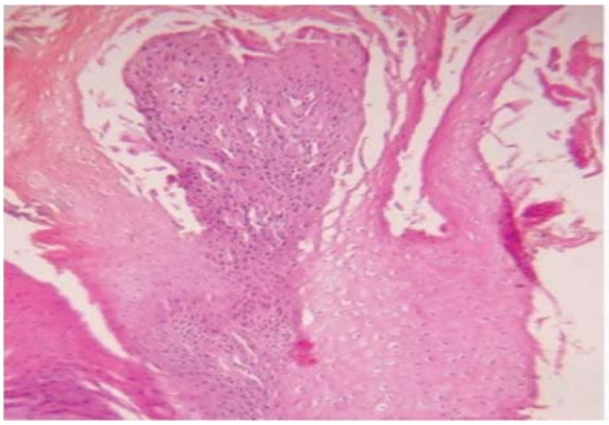

Figure 4: Koilocytes

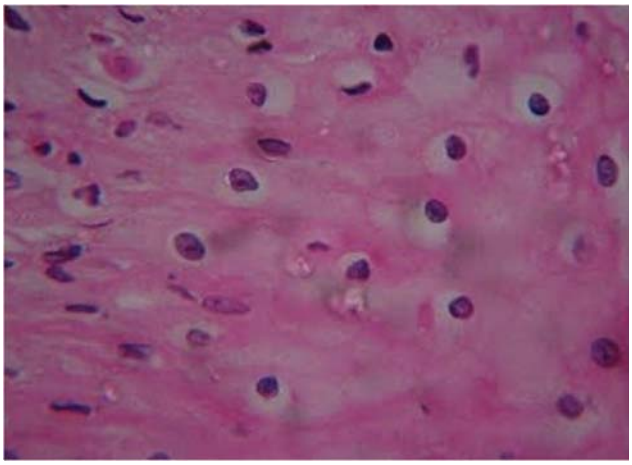

Figure 5: Koilocytes with perinuclear clearing

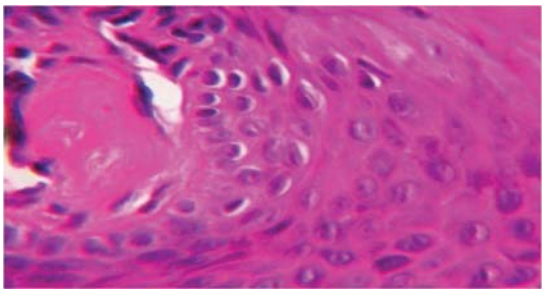

Figure 6: Focal areas showed keratin formation surrounded by koilocytes

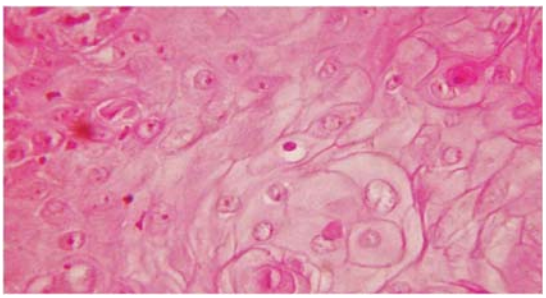

Figure 7: Altered epithelial cell with vesiculated nuclei

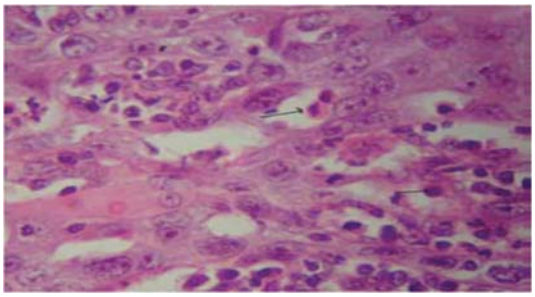

Figure 8: Binucleation

to 50% in India. The prevalence of HPV in OSCC also shows regional variation. It has been reported as 33.6% in Eastern India, 67% in South India and 15% in Western India. Chocolatewala *et al*. reported a prevalence of 17.6–41.8% for HPV16 and 0–47.3% for HPV18 in OSCC cases [21].

At present time, HPV status is not reported in the histopathological diagnosis routinely of OSCC. As there is a growing body of evidence that HPV as an aetiological factor in oral cancers, it's our opinion that the status must be incorporated into the pathology report. This will allow for possible targeted therapy in the future for patients with OSCCs.

CONCLUSION

In this study, there was a significant high prevalence of koilocytosis – 45% in OSCCs. Thus, the study points the use of light microscopic feature koilocyte for predicting HPV infection in OSCC, and this has to be incorporated in histopathological reporting of OSCC.

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