Unusual presentation of more common disease/injury

Oral verrucous carcinoma – a misnomer?

Immunohistochemistry based comparative study of two cases

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Summary

The clinico-histopathological diagnosis of oral verrucous carcinoma (OVC) is often exclusionary and extremely difficult. Distinction from the classical oral squamous-cell carcinoma (OSCC) is a frequent problem for both clinicians and pathologists because of the extensive nature of the lesion mimicking an invasive cancer. Immunohistochemistry in this case provides a platform for studying distinct molecular mechanism by variation in expression of protein markers. In this study, the authors have attempted to differentiate OVC (case no 1) and OSCC (case no 2) by studying the expression pattern of some well-known tumour marker proteins (vascular endothelial growth factor, matrix metalloproteinases 2 and 9, superoxide dismutase 2 and nicotinamide adenine dinucleotide phosphate quinone oxidoreductase 1). A distinction in expression of these proteins provides a clear understanding that OVC do not show characteristics of a classical carcinoma and may be regarded as a misnomer. This may also provide a better guide for clinicians to differentiate between these two.

BACKGROUND

Verrucous carcinoma (VC) was defined by Ackermann in 1948 (also known as Verrucous carcinoma of Ackermann or Ackermann's tumour).¹ The term 'verrucous' was applied for lesions showing a keratotic exophytic surface composed of sharp or blunt epithelial projections with keratin-filled invaginations (plugging), but without obvious fibrovascular cores. The histological features of VC, for example, verrucous surface and 'elephant feet'-like downgrowth seeming to compress the underlying connective tissue and typically showing minimal or no cytological atypia, are widely known. The oral cavity is the most common site of occurrence. In addition, it is known to occur in the larynx, pyriform sinus, oesophagus, nasal cavity and paranasal sinuses, external auditory meatus, lacrimal duct, skin, scrotum, penis, vulva, vagina, uterine cervix, perineum and the leg. This tumour is predominantly seen in males over the sixth decade.²⁻³ In terms of tumour biology, oral VC (OVC) is distinct in its slow growth and ability to become locally aggressive if not treated appropriately. However, even with local tumour progression, it is intriguing that regional or distant metastasis is rare. OVC has a characteristic gross appearance and strongly associated with the chronic use of tobacco or with the practice of chewing betel nuts. These lesions are almost always large, exophytic, soft, fungating, slow-growing neoplasms with a pebbly mamillated surface. Though this is the most common presentation of OVC, it may not always appear as the typical warty exophytic lesion described in the literature.⁴

Presently, there are mounting evidences which focuses mainly on the non-carcinomatous features of VC. However, there are several other studies which do not rule out the cancerous propensity of VC. A 'veruciform' lesion may show conventional invasive pattern. The latter represents an 'oral squamous-cell carcinoma (SCC) with an

exo-endophytic growth pattern'. Often, the invasion can be lacking in incisional biopsies and it is not possible to exclude an underlying conventional carcinoma. Distinction from classical squamous-cell carcinoma is a frequent problem especially for clinicians because of the extensive nature of the lesion mimicking an invasive cancer.⁵ Some molecular studies previously done have confusing results; where one study shows by flow cytometry that VC is a diploid lesion and⁶ on the contrary, another study shows the aneuploid nature of VC.⁷ In this case, it is to be mentioned that squamous-cell carcinoma showed aneuploidy and genomic instability.

In this current scenario, we have put forward the importance of immunohistochemistry as a confirmatory test to showcase the carcinogenic status of VC. The hallmarks of carcinogenesis are neoangiogenesis and degradation of the extracellular matrix, which helps in tumour growth, survival and metastasis of neoplastic cells. In this process, vascular endothelial growth factor (VEGF) is regarded as one of the major contributing factor in angiogenesis whereas the matrix metalloproteinases (MMPs) (a family of zinc-containing endopeptidases) causes degradation of various components of the extracellular matrix and helps in metastasis. VEGF is a potent mitogen and chemoattractant for endothelial cells and induces the release of MMP-2, MMP-9 which, in turn, regulates the angiogenic switch that can occur very early in some cancers, even before malignant progression, with increased vessel density seen in precancerous lesions.⁸⁻⁹ On the other hand, superoxide dismutase (SOD) and nicotinamide adenine dinucleotide phosphate: quinone oxidoreductase 1 (NQO1), a cytosolic enzyme detected mainly in epithelial cells catalyses the two-electron reduction of quinone compounds and prevents the generation of reactive oxygen species, thus protecting cells from oxidative damage¹⁰ and carcinogenesis,

Figure 1 Case no 1: presenting oral verrucous carcinoma (OVC) in the left buccal mucosa extending towards attached gingival.

so a high expression of the enzymes has been observed in various tumour progressions indicative of increased oxidative stress.

From the knowledge based upon our previous comparative staining of different precancerous and cancerous states, we have shown that these specific markers provides a clear picture of cancerous propensity of a precancerous state.¹¹ This study presents two well-defined cases of OVC and oral SCC (OSCC), respectively and detection of the level of protein expression (VEGF, MMPs, NQO1 and SOD) in the tissues obtained from incisional biopsies, OSCC serving as a positive control in our study.

CASE PRESENTATION

Case 1

A 35-year-old female patient reported to the out-patient department of Oral Pathology, Dr R Ahmed Dental College and Hospital, Kolkata, West Bengal, India, with a complaint of pain in a whitish growth in the left side of lower jaw. The patient had these symptoms for the last 3 months. She gave a personal history of chewing betel quid for more than 20 years. She denied any other significant medical issue. On intraoral examination, the oral hygiene status of the patient was poor. A 3×2 cm white keratotic cauliflower-like growth was present on the left buccal mucosa extending towards the attached gingiva. The lesion extended from the lower left canine to retro-molar area (figure 1). The surface of the lesion was corrugated and highly keratinised. On palpation, margins of the lesion were well-delineated and the whitish surface covering could not be scraped off. The ipsilateral submandibular lymphnodes were palpable.

An incisional biopsy was taken including the margin of the lesion under local anaesthesia and the specimen was histopathologically and immunohistochemically evaluated

for the expression of VEGF, MMP-2 and -9, NQO1 and SOD. H&E stained sections showed hyperplastic stratified squamous epithelium with marked acanthosis and downgrowth of bulbous rete ridges into the connective tissue with intact basement membrane. Marked parakeratinisation with parakeratin plugging into the epithelium was noted. The epithelial cells showed normal intercellular junctions with no evidence pleomorphism or hyperchromatism. The underlying fibro-collagenous connective tissue showed moderate infiltration of chronic inflammatory cells.

Case 2

A 45-year-old female patient reported to the out-patient department of Oral Pathology, Dr R Ahmed Dental College and Hospital, Kolkata, West Bengal, India, with a complaint of swelling in the right lower half of face for the last 2 months. She complained of pain, discomfort and inability to eat. She had a personal history of chewing betel quid three to four times a day for the last 20 years. She denied any other significant medical issue. On intraoral examination, an ulcerated lesion was present in the right lower posterior region in relation to the lower right molars. The lesion extended towards the buccal mucosa with detachment of the gingiva from the tooth surface. On palpation, the lesion extended into the buccal mucosa and the margins of the lesion were indurated (figure 2). An incisional biopsy was taken under local anaesthesia and was histopathologically and immunohistochemically evaluated for expression of VEGF, MMP-2 and -9, NQO1 and SOD. H&E stained sections revealed the presence of hyperplastic stratified squamous epithelium with break in the basement membrane and invasion of neoplastic epithelial cells in sheets, into the connective tissue. The neoplastic epithelial cells showed formation of islands with central necrosis.

Figure 2 Case no 2: presenting ulceration or lesion in the lower right molar region which was later confirmed as oral squamous-cell carcinoma.

Numerous epithelial and keratin pearls can be seen in the fibrovascular connective tissue. Moderate infiltration of chronic inflammatory cells was also noted.

INVESTIGATIONS

Both the patients were subjected to routine haemogram, which was within normal limits.

TREATMENT

In case 1, excision of the lesion was performed and evaluated histopathologically confirming the diagnosis of the incisional biopsy. Case 2 was referred to the oncology department for further treatment.

OUTCOME AND FOLLOW-UP

Figure 3 shows differential staining of the tissue sections obtained from (case 1) OSCC and (case 2) OVC patients, respectively. Immunohistochemical staining was performed using Novolink Max Polymer detection system (Novocastra, Newcastle Upon Tyne, UK) kit. Strong expression of VEGF was observed in the neoplastic epithelial islands and adjoining connective tissue but sparing the keratin pearls (figure 3A) whereas compared to this in OVC, a focal weak immunolocalisation was observed in the basal cells. A strong immunopositivity of MMP-9 especially in the surface parakeratocytic layer of stratified squamous epithelium and around the extracellular matrix of the blood vessels and a diffused distribution along the connective tissue was strongly suggestive of metastasis (figure 3A). On the other hand, mild localisation of MMP-9 in OVC strongly suggests non-carcinomatous features. MMP-2 was not found to be considerably expressed throughout any part of both the tissues, a weak expression can be observed only under high magnification in case of OVC (figure 3B). A moderately positive NQO1 expression throughout the thickness of the surface epithelium and

neoplastic epithelial islands were present (figure 3C). Weak NQO1 expression was observed in the parabasal cell layers of OVC (figure 3C). A focal immunopositivity of SOD in the neoplastic epithelial islands was noted and can be better observed under high magnification (figure 3B). A diffuse or weak expression of SOD was noted in the basal-cell layer and the juxta-epithelial connective tissue zone (figure 3B).

DISCUSSION

In India, the use of smokeless tobacco is found in different forms, sometimes it is used with slaked lime (Khaini) and sometimes fine-cut tobacco leaf is used with betel quid. These forms are chiefly seen to be chewed and held in the buccal/labial vestibule habitually till it completely disintegrates and discarded. Most of the habitual chewers show varying degree of keratinisation/keratotic white patches in those particular areas where they habitually hold the smokeless tobacco. These areas, when histopathologically evaluated reveal varying grade of epithelial dysplasia along with surface keratinisation of variable thickness termed as epithelial hyperplasia.

Previously, various case studies have been reported focusing on the occurrence of OVC in different parts of the world.^{2 12 13} The basic contradiction here is, with leukoplakic patches of long-standing history of chewing smokeless tobacco forms, the chances of epithelial dysplasia is more and at times may even present as carcinoma in situ or frank squamous-cell carcinoma, whereas VC on the other hand seldom show features of epithelial dysplasia/atypism, associated with broad and bulbous rete pegs with generally intact basement membrane. The hallmark for diagnosis of OVC still remains the presence of parakeratinisation plugging.⁴

It is observed that long-standing leukoplakia with cellular atypism have much higher propensity to turn into

Figure 3 Comparative immunolocalisation of tumour marker proteins in serial sections obtained from oral squamous-cell carcinoma (case 1) and oral verrucous carcinoma (case 2). (A) Vascular endothelial growth factor (VEGF) and matrix metalloproteinase 9 (MMP-9). (B) Matrix metalloproteinase 2 (MMP-2) and superoxide dismutase (SOD). (C) Nicotinamide adenine dinucleotide phosphate quinone oxidoreductase 1 (NQO1). Magnification: (A) and (C), 10 $\times$; B 40 $\times$.

invasive squamous-cell carcinoma but VC may remain without invasion for similar time period which basically speaks about its non-aggressive nature.

Verrucous hyperplasia is similar in appearance to VC (Ackermann's tumour), a similar lesion has been described as Florid oral papillomatosis. Hansen *et al* in 1985 reported a further similar form, which they called proliferative verrucous leukoplakia. Verrucous hyperplasia is usually diagnosed in patients over the age of 60 years. All areas of the oral cavity can be affected, primarily the gingival, alveolar mucosa, tongue and floor of the mouth. Epithelial dysplasia is detected in 70% of such cases. On the other hand, 2–20% of all oral carcinoma is verrucous type. This type is observed primarily in southern/southeast Asia. VC is characterised by expansive growth but relatively little invasion. Clinically, the tumour appears as a white papillomatous mass with deep folds and grooves. The prognosis is better than squamous-cell carcinoma. Squamous epithelial dysplasia is characterised by a precancerous lesion by cellular atypism and loss of normal epithelial maturation and stratification.¹⁴

The epithelial dysplasia encompasses a broad range of changes, the more pronounced and more frequent lesions, the severity of the dysplasia increases. When considering the severity of dysplasia, there is relative subjectivity with regard to the recognition and interpretation of the significance of minor or more pronounced changes.¹⁴ Cellular atypia may also occur during the course of inflammatory reactions. Nevertheless, any degree of epithelial dysplasia, even a mild degree may indicate an elevated risk of malignant transformation.¹⁴

From another aspect, the tumour protein markers are now gaining importance in regards to detection of molecular progression and invasiveness of various cancers. These are also used extensively in clinical diagnosis of various cancers such as breast carcinoma, bladder cancer, etc. Among the well-known tumour markers, VEGF and MMPs (MMP-2 and -9) are acclaiming their importance. The interplay of these proteins is now shown to control two of the vital hallmarks of carcinogenesis – the neoangiogenesis and metastasis of tumour cells. Again, a well-known antioxidant enzyme, SOD and upcoming NQO1 enzyme provides the picture of oxidative stress status of the tissue. Thus immunohistochemical localisation of these proteins in cancerous tissue provides a platform to observe the interplay or interconnectivity for progression of the disease process. Therefore, it was thought to design

the study with a comparative evaluation of these proteins in squamous-cell carcinoma of the oral cavity with that of OVC to see its correlation. To our surprise OVC did not show similarity in expression of the above mentioned proteins with that of the OSCC, rather proteins like VEGF, MMP-2 and MMP-9 showed a basal level of expression. However, high expression of NQO1 and SOD suggested a high-oxidatively stressed tissue conditions.

The present case study might be for the first time, provide a comparative evaluation of OSCC and OVC on basis of expression of some tumour marker proteins. Observation suggests a clear diversion from the present concept of understanding VC and also attempts to explain the non-carcinomatous nature of VC and supports the histopathological findings of non-similarity with features of OSCC. However, much extensive studies using various other markers such as proteins related to basal lamina (as no disintegration or epithelial invasions are noted in VC) are required. No doubt, these markers can obviously now can guide the pathologists with proper and a less confusing diagnosis of VC.

Based upon this immunohistochemical study; can we really identify or label these tumours as carcinomas? Or we can wind up claiming that these white papillomatous mass are verrucous hyperplasias clinically and that VC is rather a misnomer.

Learning points

- ▶ OVC do not show features of the classical squamous-cell carcinoma
- ▶ Difference in expression patterns of protein tumour markers may serve as a basis of differentiation between OVC and OSCC
- ▶ VEGF, MMP-9 may serve as two promising markers.

Competing interests None.

Patient consent Obtained.

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