J. Maxillofac. Oral Surg. (July–Sept 2015) 14(3):599–604
DOI 10.1007/s12663-014-0715-8

RESEARCH PAPER

Reliability of Incision Biopsy for Diagnosis of Oral Verrucous Carcinoma: A Multivariate Clinicopathological Study

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Received: 11 March 2014 / Accepted: 7 October 2014 / Published online: 25 October 2014

Abstract
Introduction Studies have reported 20 % of conventional squamous cell carcinoma in patients with verrucous carcinoma (VC), later these cancers were termed as hybrid VC. It is important to distinguish both while planning treatment since hybrid VC requires addressing regional lymphatics in addition to respective surgery. Information on odds of missing the foci of invasion on routine incision biopsy might be useful in this regard.
Patients and Methods Records of all the patients surgically treated for oral cancer from Jan 2010 to Oct 2013 in a Tertiary Cancer Centre was analyzed. Patients diagnosed with primary VC or Verrucous Hyperplasia on incision biopsy were included in the study. Proportion of patients undiagnosed for invasive component on incision biopsy was calculated, multivariate analysis of the sample was performed to find associated cofounders.
Results Fifty-five patients who reported with the diagnosis of VC (n = 53) or Verrucous Hyperplasia (n = 2) on incision biopsy were included in the study. Twenty-seven were diagnosed as VC and 28 as hybrid VC after excision. This corresponded to 51 % (n = 28) of cases missing invasive component on incision biopsy. VC was significantly more commonly seen in lip and in buccal mucosa, hybrid VC was more commonly seen in tongue and gingiva and this association was statistically significant ($p = 0.031$) in our study.
Conclusion Incision biopsy is extremely unreliable to diagnose and differentiate oral Hybrid VC from VC or Verrucous Hyperplasia. Caution is required while planning treatment of these patients regarding possibility of presence of conventional squamous cell carcinoma within these tumors.

Keywords Oral hybrid carcinoma · Verrucous carcinoma · Incision biopsy · Diagnosis

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Introduction

In 1948, Ackerman [1] introduced the term "Verrucous Carcinoma" (VC) to describe a variant of squamous cell carcinoma of the oral cavity. VC is a warty variant of squamous cell carcinoma characterised by predominant exophytic overgrowth of well differentiated keratinizing epithelium having minimal atypia with locally destructive pushing margins at its interface with underlying connective tissue [2].

Literature showed controversies regarding terminologies used to describe these tumors for clinical diagnosis, histopathological diagnosis and management. VC was

inconsistently termed with variety of names such as Oral Florid Verrucosis, Verruca Acuminata, Verrucous Squamous Cell Carcinoma, Oral Florid Papillomatosis and Papillomatoses Mucosae Carcinoides. By the 1970s, they were consistently termed as "VC". However; in 1980, Shear and Pindborg [3] observed that these lesions are difficult to be differentiated from verrucous hyperplasia on clinical examination alone. Verrucous hyperplasia is described as exophytic overgrowth of well differentiated keratinizing epithelium that is similar to VC but without destructive pushing border at its interface with underlying connective tissue.

Other differential diagnosis of VC is proliferative verrucous leukoplakia (PVL), and pseudoepitheliomatous hyperplasia. True incidence pattern of VC was not possible due to its rarity, impossibility of differentiating them on incision biopsy from the intermediate varieties consisting of verrucous hyperplasia or invasive squamous cell carcinoma and the inconsistent terminologies used in the literature. Often all of these existed synchronously or progressed from their precursors. Thus the cases treated with radiotherapy were not reported due to unavailability of resection specimen.

Medina et al. [4] in 1984 reported coexistence of foci of SCC in VC for the first time. Incidence of these lesions was 20 % in their clinicopathologic study of 104 cases. Slootweg [5] in his series reported 37 % of synchronous or metachronous SCC. Initially VC was treated by either radiotherapy or surgery as suggested by Ackerman until anaplastic transformation of VC was reported. Increasingly surgery was popularised for the management of VC [6–9], however; the anaplastic transformation was later attributed to synchronously existing much poorly differentiated carcinoma in those lesions [10]. Surgery was more preferred treatment because of better local control rates; the local control rates of surgery was 85 % compared to radiotherapy which was <50 %. This lead to availability of complete resection specimen and increased reporting of synchronous SCC.

A hybrid VC is a non-verrucous SCC (squamous cell carcinoma) that arises synchronously with the VC. There is a profound difficulty in diagnosing these lesions which show subtle differences in clinical appearances.

The management of VC is different from hybrid VC, either of lesions requires surgical excision with adequate margin but VC does not require regional lymphatic clearance, whereas, hybrid VC is managed similar to conventional SCC [11].

Unfortunately accurate diagnosis of these lesions is possible only after definitive surgery for primary disease since the entire specimen is available for processing. The time required to process specimen, the second surgery to address neck in hybrid VC cases and the duration required for operative site healing can be determinant in cases requiring adjuvant therapy. Further, any postoperative complications such as infection or reconstructive flap failure can prolong this crucial period. Inflammatory cervical lymphadenopathy is common in these patients [12, 13], current imaging modalities are not useful to detect occult metastasis. Sentinel node biopsy mentioned in the recent publications may be promising such clinical scenarios [14], but clinical expertise of these methods are not available uniformly, neither their validation can be yet declared as a standard of care [15].

As a result, second surgery addressing neck is required in few cases finally diagnosed as hybrid VC; further delay in adjuvant therapy is possible rendering it inefficient in neck positive cases. Information on reliability of incision biopsy for the diagnosis of foci of SCC in VC may be useful to approve or disapprove selective neck dissection in cases diagnosed as VC on incision biopsy.

Patients and Methods

Records of all the patients surgically treated for oral cancer from Jan 2010 to Oct 2013 in a Tertiary Cancer Centre was analysed retrospectively (IRB exempted for retrospective nature of the study), Patients diagnosed with primary VC or Verrucous Hyperplasia on incision biopsy were included in the study.

The institute follows universally accepted protocol for validation of biopsies. Superficial biopsies are advised to be repeated by pathology team and reporting is differed unless the specimen submitted fulfils the criteria for reporting of incision biopsies; biopsy is considered valid only when enough depth to include sufficient amount of connective tissue is achieved along with an adjacent normal mucosa and the tumor. The tissue is transferred to 10 % formalin in 1:10 ratio by volume immediately following the biopsy. Biopsies on patients with previous surgery or radiation for oral cancer were excluded from the study. Patients with extensive lesions in whom reconstructive options required access to neck, underwent elective neck dissections along with the patients showing other risk factors such as extensive bone and skin involvement. However, neck dissection was limited to level I–III in these cases, in tongue cancer cases; level IV dissection was done along with level I, II and III.

All the patients were staged based on UICC TNM staging; patients in whom final histopathology revealed hybrid VC but neck dissection was not done were included in a separate category (Nx).

Results

The current study consisted of 55 patients diagnosed as VC or Verrucous Hyperplasia on incision biopsy after

Table 1 Demographic details of patients with VC and hybrid VC

	VC (27)	Hybrid VC (28)
Age	21–76 years, mean 52 years	38–78 mean 55.39
Sex		
Male	16	14
Female	11	14
Site		
Buccal mucosa	16	14
Gingiva	0	6
Tongue	6	7
Lip	5	1
T stage		
T1	4	5
T2	16	18
T3	5	2
T4	2	3
N stage		
N0	21	21
N2b	0	2
Nx[a]	6	5
Presence of associated synchronous second primary verrucous hyperplasia		
Present	3	1
Absent	24	27

[a] Patients in whom neck dissection was not done

excluding patients with history of treatment for oral cancer. Among these patients, two patients were diagnosed as Verrucous Hyperplasia, and 53 patients were diagnosed as VC on incision biopsy. One patient reported with a synchronous second primary tumor, both lesions were diagnosed as VC on initial biopsy, however; final histology revealed hybrid VC in both sites.

Overall, among 55 patients; 28 (51 %) patients showed hybrid VC and 27 (49 %) showed VC on final histopathology. Of the patients diagnosed with hybrid VC; 22 patients had well differentiated SCC component, 5 patients moderately differentiated and 1 patient poorly differentiated SCC component. Invasive foci were missed in 51 % of cases on incision biopsy.

Demographic Data of the Analysed Sample

Age of the patients ranged from 21 to 78 years, mean 53.7 years. Twenty-five (45.4 %) patients were females and 30 (54.5 %) were males. Thirty (54.5 %) patients had tumor over buccal mucosa, 13 (23.6 %) patients on tongue, 6 (10.9 %) patients on lip and 6 (10.9 %) over the gingiva. All the tumors clinically presented as a proliferative exophytic growth. Nine (16.3 %) patients presented tumor in

Table 2 Association between Final histology and site

Site	VC	Hybrid VC
Lip	5	1
Buccal mucosa	16	14
Tongue	6	7
Gingiva	0	6
	p value 0.031	

Table 3 Association between final histopathology and T-stage

T stage	VC	Hybrid VC
T1	4	5
T2	16	18
T3	5	2
T4	2	3
	0.638	

T1 stage, 34 (61.8 %) in T2 stage, 7 (12.7 %) in T3 stage and 5 (9 %) in T4 stage Forty-two (76.3 %) patients had neck N0, 2 (3.6 %) N2b and 11 (20 %) patients did not undergo neck dissection (Nx). Demographic details of patients with VC and hybrid VC separately are listed in Table 1. Statistical analysis of data revealed following results: VC often presented over lip and buccal mucosa and hybrid VC was more often seen over tongue and gingival ($p = 0.031$) (Table 2).

VC often presented in T2 and T3 stages and Hybrid VC was more often seen in second stage. However, this association was not statistically significant ($p = 0.638$) (Table 3).

Buccal mucosa was more often involved in hybrid VC; adjusted odds ratio (OR) 4.88 [95 % Confidence Intervals (CI) 6.47, 51.17] ($p = 0.186$) and for tongue; OR 5.91 (95 % CI 0.51, 68.24) ($p = 0.155$). However, it was not statistically significant (Table 2).

Histopathological finding of hybrid VC was more often in T1 stage cancer patients when compared to T4 stage of cancer patients, OR 1.34 (0.10, 17.46) ($p = 0.825$) (Table 4).

A total of four patients presented with synchronous second primary verrucous hyperplasia. Patients with synchronous second primary verrucous hyperplasia showed primary tumor often over lip, adjusted OR 1.00 (95 % CI 0.05, 21.91) ($p = 1.000$) and less often over buccal mucosa, (0.30 95 % CI 0.01, 7.26) (0.458) and tongue; OR 0.55 (95 % CI 0.03, 11.69) ($p = 0.700$). However it was not statistically significant (Table 5).

Synchronous second primary verrucous hyperplasia was less frequent in patients with neck status N0, OR 0.36 (95 % CI 0.04, 3.73) ($p = 0.393$) (Table 5).

Table 4 Multivariable analysis explaining the association between hybrid VC with site, different T-stage

Factors	N	Hybrid VC; n (%)	Odds ratio (95 % confidence intervals)	*p* value
Site				
Lip	6	1 (16.7)	1.00	
Buccal mucosa	30	14 (46.7)	4.88 (6.47, 51.17)	0.186
Tongue	13	7 (53.8)	5.91 (0.51, 68.24)	0.155
Gingiva	6	6 (100)	–	–
T-stage				
T1	9	5 (55.6)	1.34 (0.10, 17.46)	0.825
T2	34	18 (52.9)	0.94 (0.11, 8.04)	0.955
T3	7	2 (28.6)	0.48 (0.03, 6.05)	0.550
T4	5	3 (60.0)	1.00	

Table 5 Multivariable analysis explaining the association between cases with synchronous Verrucous Hyperplasia to site and neck status

Factors	N	Synchronous verrucous hyperplasia; n (%)	Odds ratio (95 % Confidence intervals)	*p* value
Site				
Lip	6	1 (16.7)	1.00 (0.05, 21.91)	1.000
Buccal mucosa	30	1 (3.3)	0.30 (0.01, 7.26)	0.458
Tongue	13	1 (7.6)	0.55 (0.03, 11.69)	0.700
Gingiva	6	1 (16.7)	1.00	0.825
Neck status	11	2 (18.2)	1.00	
Nx	42	2 (4.8)	0.36 (0.04, 3.73)	0.393
N0	2	0 (0.0)	–	–
N2b				

Discussion

VC represents 2–12 % of oral cancers, occurring mainly in older men; mean age of presentation is 69 years [7]. However some studies have reported equal sex distribution [16], and some with female predominance [6, 12].

It is strongly associated with use of chewable form of tobacco and betel nut [17]. The most common site of occurrence is buccal mucosa followed by mandibular alveolar ridge and gingiva [18].

VC is commonly seen in lip and buccal mucosa. However, current study showed such morphologically appearing lesions on gingiva and tongue initially diagnosed as VC on incision biopsy often consisted of invasive foci of SCC in them (*p* value 0.031, Table 2).

The description of VC by Ackerman subsequently lead to its identification and reporting. All the other terminologies previously used for such lesions were discontinued over a period of time. However, the difficulty of identifying VC from verrucous hyperplasia was pointed by various researches [3]. Besides the focal basal cell nuclear hyperchromatinism and benign nature of the lesion, distinction of verrucous hyperplasia was not possible from VC on cytological features alone [11, 19]. Close communication between the clinician and the pathologist was always necessary to differentiate them from each other. Multiple biopsies were often needed for appropriate diagnosis. Such situations lead to multiple biopsies in our sample. Nevertheless the biopsy was not considered valid unless it fulfilled the universally accepted protocol for reporting. Shear and Pindborg [3] suggested that verrucous hyperplasia can be best differentiated from VC in biopsies taken from the margins of the tumor. They described that within verrucous hyperplasia, verrucous processes and great part of the hyperplastic epithelium are superficial to adjacent normal epithelium, whereas in VC, verrucous processes are superficial but broad rete processes extend into the connective tissue deeper than adjacent normal epithelium.

Slootweg [5] concluded that verrucous hyperplasia was probably a morphological variant of VC. Further, he described the stages of pathological progression of verrucous hyperplasia to VC and proposed that the existence of VC represents a premalignant change of an entire mucosa. This theory was based on Slaughter's [20] field cancerization concept. Batsaki et al. [10] placed them into 4 stages—the clinical flat leukoplakia without dysplasia, verrucous hyperplasia, VC, and conventional squamous cell carcinoma. In order to attend to this phenomenon we attempted to analyse coexisting second primary verrucous hyperplasia as a variable. Patients with primary established cancer (VC or hybrid VC) at lip often had a history of multiple synchronous or metachronous verrucous hyperplasia. Although not statically significant this association was less common in patients with primary cancer on buccal mucosa and tongue. Medina et al. [4] in their study of 104 cases of VC stressed on the surgical management of VC since 20 % of the tumors showed coexistence of less differentiated carcinoma in their cohort. Observation of the data of our institute showed higher occurrence of hybrid VC to VC (1.5–4.5 % for VC and 3.38–6.4 % for Hybrid VC).

Recent genetic and molecular studies focused to distinguish VC and conventional SCC. VC unlike conventional SCC exhibited cells with S-phase confined to basal layers. Flow cytometry confirms that it is a diploid lesion unlike SCC which is an aneuploid lesion [21].

CD44v9 was detected in VC more frequently than conventional SCC which possibly explains the low incidence of metastasis to lymphatics [22]. The immunohistochemical expression of p16 was low in dysplasia and

squamous cell carcinoma, whereas it was high in VC. This indicates the possibility of HPV association to VC [23]. HPV was identified in 85 % of laryngeal VCs [24].

There was a great variation in the expression of Ki-67 and p53 in VC in comparison to SCC. Expression of Ki-67, p53 was significantly higher in SCC [23], but VC showed higher expression of Ki-67 and p53 in comparison to verrucous hyperplasia. This may serve as a useful diagnostic tool in difficult cases [25].

Valuable information has been gathered by all these studies, but until now there is no method to derive these results into clinical applicability. Cost of these investigations limit their routine use in developing and underdeveloped countries.

Controversies in treatment planning of these tumors are likely when establishment of diagnosis without excision is not possible. Although, surgery is preferred certainly over radiation, this choice is influenced by superior cure rates with surgery rather than reports of anaplastic transformation of VC.

VC irrespective of size does not metastasize to regional lymphatics. However, inflammatory lymph nodal enlargement is frequent in these patients [12, 13]. Hybrid VC metastasises to regional lymphatics [11]. Our study emphasizes on difficulty of identification of these lesions on initial biopsy. Patients without sophisticated reconstructions might be re-operated for neck nodes easily after analysis of entire specimen, but the cases of VC often present wide areas of dysplasia and extensive reconstructions are not uncommon. Re-surgery of neck nodes may be complicated with microvascular flaps in situ along with the vascular anastamosis in the neck. Authors observed that 51 % of incision biopsies failed to identify SCC component of hybrid VC. Repeated biopsies for differentiating verrucous hyperplasia from VC seems justified since plan of excision may differ (VC requires adequate margin clearance whereas verrucous hyperplasia may be simply excised) but diagnosis of VC would not usually follow with repetition of biopsy for detection of invasive component. Delay in initiation of adjuvant therapy as a result of multiple procedures in the patients undiagnosed for invasion on initial biopsy is considerable. Our data showed two patients with an occult N2b neck status. Our sample was not sufficient to establish the risk of such situation. Invariably selective lymph nodal clearance for final staging in patients requiring extensive reconstructions was often performed. Quantification of verrucous areas and SCC areas in hybrid VCs will be beneficial in planning adjuvant therapy. Diagnosis of hybrid VC is not reliable on incision biopsy. Risk of occult metastasis in patients undiagnosed with invasive component on initial biopsy persists. Authors intend to caution regarding these possibilities through this study.

Acknowledgments Authors acknowledge Dr. Sannapaneni Krishnaiah (Biostatistian) for professional help in statistics.

Conflict of interest None.

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