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ORIGINAL ARTICLE

Evaluation of cannibalistic cells: a novel entity in prediction of aggressive nature of oral squamous cell carcinoma

DEEPA JOSE[1], DEEPA R MANE[1], UMA DATAR[1], SIDHRAMESH MUTTAGI[2], SEEMA HALLIKERIMATH[1] & ALKA D KALE[1]

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Abstract

Objective. Cellular cannibalism is a distinctive morphologic feature exclusively seen in aggressive malignancies and is defined as a large cell enclosing a slightly smaller one within its cytoplasm. This phenomenon has been illustrated in several malignancies and is correlated well with the degree of anaplasia, invasive and metastatic potential of tumor cells. However, this marker has not been extensively studied and is often unnoticed during the routine histopathological assessment of Oral squamous cell carcinoma (OSCC). Thus, the aim of this research was to evaluate the presence of *cannibalistic cells* (CC) and to find if there exists any association with the aggressive nature of OSCC. ***Materials and methods:*** In total, 20 neck dissection cases of OSCC with follow-up data were included in the study. The cannibalistic cells were evaluated in the lesion tissues. Cellularity of cannibalism is graded asGrade I: < 5 cells, Grade II: 6–15 cells and Grade III:> 16 cells. The data was analyzed using Fischer Exact test. ***Results.*** Out of 20 cases, all the cases showed presence of CC, Grade I in five cases, Grade II in eight cases and Grade III in seven cases. A statistically significant relation between advanced grade of cellular cannibalism and lymph node positive status ($p \leq 0.001$) was obtained. ***Conclusion.*** Interestingly the cases with positive lymph node metastasis demonstrated Grade 3 CC.Hence, during routine histopathological examination, the search of CC can be considered as one of the important parameters to note the aggressive nature of OSCC.

Key Words: *cannibalism, cannibalistic cell (CC), oral squamous cell carcinoma (OSCC)*

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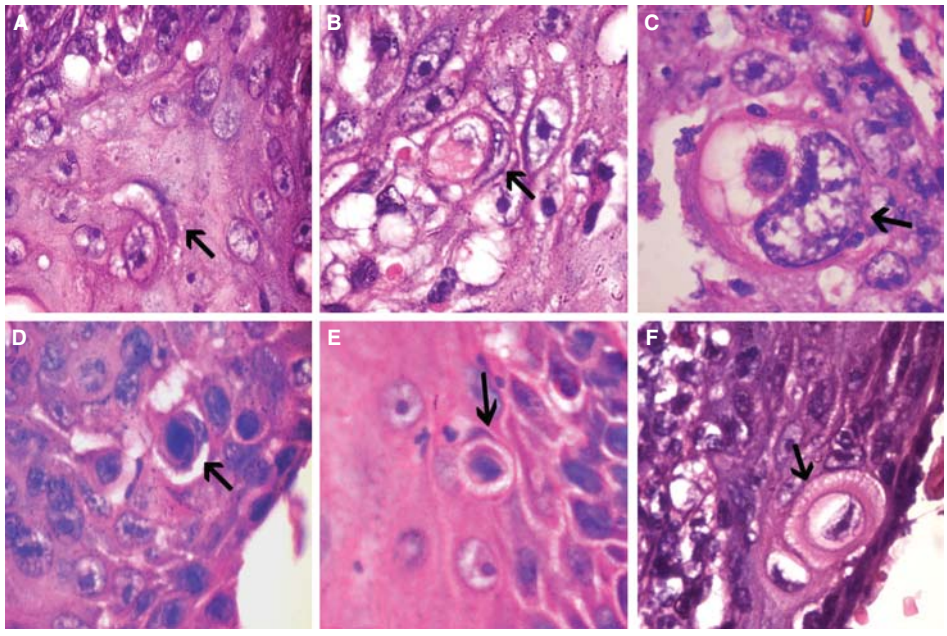

Figure 1. (A,B,C,D,E,F) Photomicrograph of H & E stained sections of Oral squamous cell carcinoma pointing cannibalistic cells (× 100).

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Introduction

Head and neck squamous cell carcinoma accounts for the sixth most common human cancer in the world [1] and in addition it holds for the third position of human tumor in some developing countries, like India, Sri Lanka, Philippines, Brazil and Pakistan. The prevalence varies depending on the geographical area and the mode of consumption of tobacco [2,3].A worldwide increase in cancer rates are anticipated from 10 million new cases in 2000 to 15 million in 2020, as estimated by the International Agency for Research on Cancer of the World Health Organization (IARC–WHO) [4]. Regardless of availability of newer diagnostic and therapeutic strategies concerning oral squamous cell carcinoma (OSCC) diagnosis and therapy, the survival rate of patients has not shown much improvement.

Molecular biology has enhanced the standard of cancer diagnosis; nevertheless,histopathological assessment of formalin-fixed biopsy tissue specimens remains the gold standard of cancer diagnosis [5]. Advanced diagnostic modalities like Polymerization chain reaction, ELISA, cell culture and immunohistochemistry have been proposed as prognosticators in carcinomas; however, these are yet to have an impact

Table I. The demographic and follow-up data of the neck dissection cases of oral squamous cell carcinoma.

Sl No	Sex	Age (years)	Habit	Site	TNM stage	Grade	L N+/−ve	Recurrence	Metastasis	Survival
1	M	48	No	Palate	T4aN1M0	WDSCC	+ve	Yes	Yes	Yes
2	F	60	Yes	Gingivobuccal sulcus	T4N2aMo	MDSCC	+ve	No	No	Yes
3	M	54	Yes	RetromolarTrigone	T4aN2bMo	WDSCC	+ve	No	No	Yes
4	F	62	No	Tongue	T3N1M0	MDSCC	+ve	No	Yes	Dead
5	M	36	Yes	Buccal Mucosa	T4aN0M0	WDSCC	+ve	No	No	Yes
6	M	52	Yes	RetromolarTrigone	T4aN1M0	MDSCC	+ve	No	No	Yes
7	M	42	Yes	Tongue	T4aN2cM0	WDSCC	−ve	No	No	Yes
8	M	38	Yes	Palate	T4N0Mo	WDSCC	−ve	No	No	Yes
9	M	90	Yes	RetromolarTrigone	T4aN0Mo	MDSCC	−ve	No	No	Yes
10	M	55	Yes	Buccal Mucosa	T4aN1M0	MDSCC	−ve	No	No	Yes
11	F	58	Yes	Gingivobuccal sulcus	T3N2aM0	WDSCC	−ve	No	No	Yes
12	M	44	Yes	Posterior mandibular alveolus	T4aN0M0	WDSCC	−ve	No	No	Yes
13	M	56	Yes	Buccal Mucosa	T3N0M0	MDSCC	−ve	No	No	Yes
14	M	65	Yes	Buccal Mucosa	T3N0M0	WDSCC	−ve	No	No	Yes
15	M	48	Yes	Gingivobuccal sulcus	T3N0M0	WDSCC	−ve	No	No	Yes
16	M	74	Yes	Buccal Mucosa	T3N0Mo	PDSCC	−ve	No	No	Yes
17	M	35	Yes	Labial Mucosa	T4N0M0	WDSCC	−ve	No	No	Yes
18	M	45	Yes	Buccal Mucosa	T3N0M0	WDSCC	−ve	No	No	Yes
19	M	47	Yes	Buccal Mucosa	T4aN2aM0	WDSCC	−ve	No	No	Yes
20	M	52	Yes	RetromolarTrigone	T4aN1M0	MDSCC	−ve	No	No	Yes

on routine clinical care [6]. Moreover, these facilities may not be feasible or affordable for all the patients, especially in developing countries [7].Therefore, in order to improve the individual management of OSCC, ahistopathological factor in routine Hematoxylin & Eosin stain with prognostic relevance is need of the hour.

Borders' grading system for categorization of OSCC into well, moderate and poor is based on degree of differentiation of the tumour cells and is the most routinely used method [7].However, since OSCC consists of a diverse cell group with different metastatic and invasive behavior, there exists a lack of relationship between these grades and the prognosis of OSCC [7]. Additional histological parameters like tumor thickness [8], lymphovascular invasion [9], perineural spread at the invasive front [10]; micro vascular density [11] and tissue eosinophilia [12] have been explored and are described as major risk factors that adversely affect the prognosis of patients.On the other hand, not much attention has been paid to the cellular aspect of histology of tumor which can be of relevance in terms of grading the tumor and its aggressiveness. One such feature is *cellular cannibalism* (CC), which can be routinely seen but often neglected for its importance.

Cellular cannibalism is a distinctive morphologic feature exclusively seen in aggressive malignancies and is defined as a large cell enclosing a slightly smaller adjacent tumor cell within its cytoplasm for its survival. The presence of these cells may denote the general growth and behavior of tumor [13].This phenomenon has been illustrated and found its association with the degree of anaplasia, invasiveness, aggressiveness and metastatic potential of various malignancies such as in breast cancer [14], malignant melanoma [15], giant cell carcinoma of lung [16],gall bladder carcinoma, endometrial stromal carcinoma [17],malignant thymoma [18], etc. Nevertheless, this vital marker of aggressive biological behavior has not been extensively studied and is unnoticed during routine histopathological assessment of head and neck OSCC. Hence, the aim of the present research was undertaken as a preliminary work with the aim to evaluate the presence of cannibalistic cells (CC) and to find if there exists any association with the aggressive nature of OSCC.

Materials and methods

Case selection

In total, 20 neck dissection specimens of primary OSCCs between the period (November 2010 and August 2012) were included in the study. After obtaining the institutional ethical clearance, formalin fixed paraffin embedded tissue blocks of the lesion proper and lymph nodes of neck dissection

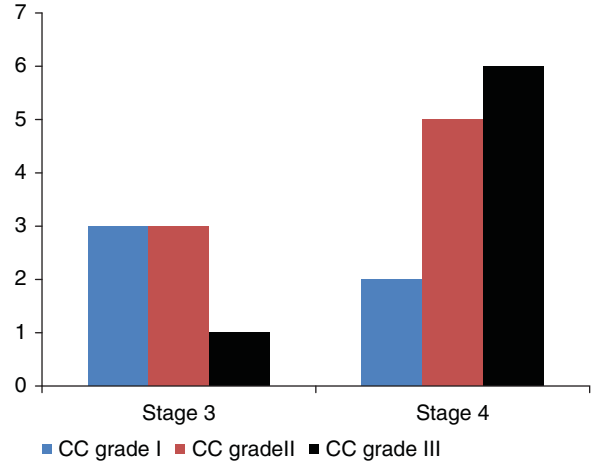

Figure 2. Bar graph representing the correlation of clinical TNM staging and CC grade.

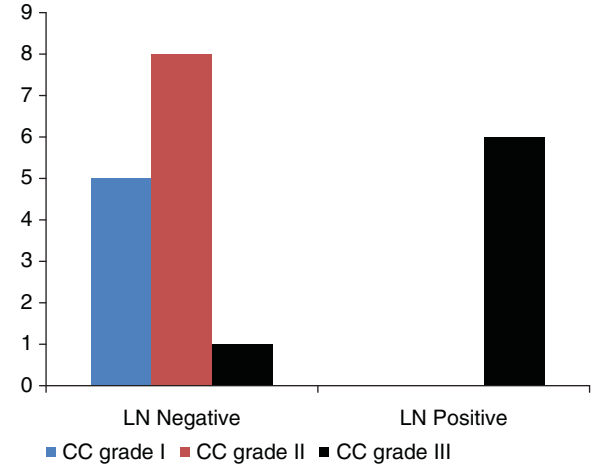

Figure 4. Bar graph representing the correlation of lymph node metastasis and CC grade.

specimens were retrieved from the archives of the Department of Oral Pathology and Microbiology. Demographic data regarding age, gender, habit history and TNM stage of all cases were noted from the records. The available follow-up data to date regarding metastasis, recurrence and survival were also collected.

Four-micrometer thick sections of lesion proper were obtained and stained with routine hematoxylin and eosin. The stained sections were analyzed further to confirm the diagnosis and were graded based on Borders' system as well, moderate, poorly differentiated OSCC and further tumor cells were evaluated for the presence of cannibalistic cells. All the obtained lymph nodes were also examined to note the presence or absence of metastatic tumor islands.

Evaluation of cannibalistic cell (CC)

A large tumor cell engulfing or engulfed another tumor cell with a crescent shaped nucleus was considered as a *cannibalistic cell* (CC) (Figure 1). Overlapping of tumor cells, dyskeratotic cells, koliocytic cells and degenerating cells were excluded during evaluation. Sections were observed with an×100 objective (oil immersion) under a light microscope (Leica research microscope) at 10 different fields, which showed maximum density of such a phenomenon. Based on the frequency of presence of *cannibalistic cells* (CC grade), it was graded as Grade I: < 5 cells, Grade II: 6–15 cells and Grade III: > 16 cells.

To ascertain the correlation between CC grade and the tumor nature and behavior, a comparison of CC grade with the clinical TNM staging, histopathology grading, lymph node metastasis, recurrence and survival were done.

Statistics

The data regarding clinical TNM staging, OSCC histopathology grade, lymph node metastasis, follow-up data and CC grade were entered in an

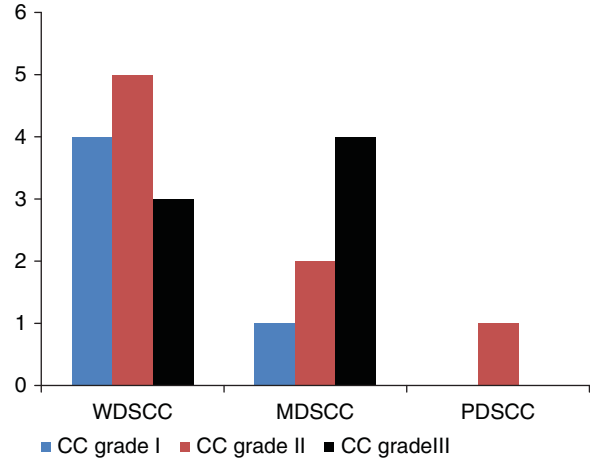

Figure 3. Bar graph representing the correlation of histopathology grading and CC grade.

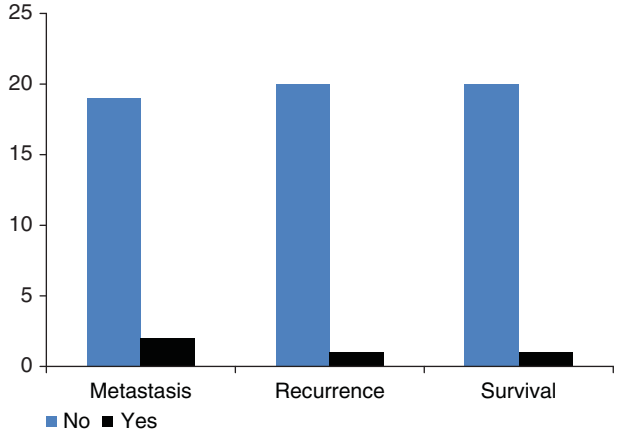

Figure 5. Bar graph representing the correlation of follow-up data and CC grade.

Excel spread sheet of SPSS software. Statistical analysis was done using Fischer Exact test.

Results

Out of 20 cases, 17 cases were males with a mean age of 57.5 ± 13.63 years and three cases were females with mean age of 60 ± 2 years. The majority of cases had tobacco as a predisposing factor and only three cases had a non-tobacco history, with trauma being the etiological factor (Table I). Predisposing etiological factors did not have much association with the number of CC (CC grade). On analyzing the patient case histories, 10 cases showed clinically positive lymph node status, amongst them five cases showed a single ipsilateral palpable and fixed lymph node, four cases with multiple ipsilateral palpable and fixed lymph nodes and only one case showed clinical evidence of metastasis in contralateral lymph node (Table I).

Clinical staging and CC grade

In the present study seven cases were in stage 3 and 13 cases in stage 4 of clinical TNM. The majority of cases of Grade III CC were found in clinical stage 4 of OSCC. Thus, with increase in tumor size and stage there was an increase in frequency of CC (Figure 2).

Histopathology grading and CC grade

On comparing the frequency of CC with various grades of OSCC, we observed the presence of such cells in all the grades. Grade I of CC was seen in five cases (four WDSCC, one MDSCC) of OSCC, Grade II was found among eight cases (five WDSCC, two MDSCC, one PDSCC) and Grade III was found among seven cases (three WDSCC, four MDSCC) of OSCC. The CC grade did not show any statistically significant correlation with the histopathological grades (Figure 3).

Lymph node metastasis and CC grade

Out of 10 cases of clinically positive lymph nodes only six cases were histologically positive with presence of tumor cells, while the other four cases demonstrated reactive hyperplasia of the lymph node. On correlating the CC grade with the positive lymph node status, surprisingly we found that all these six cases were found to be in Grade III CC and also showed a significant relation statistically ($p \leq 0.001$) (Figure 4).

Follow-up data and CC grade

On assessing the follow-up data of the cases we found that only one case had a recurrence, two cases showed metastasis and only one case was deceased. All these four cases showed a Grade III CC, but the results could not establish a statistically significant outcome (Figure 5).

Discussion

Histopathological evaluation remains the mainstay in deciding the treatment protocol and prognosis of surgical resected individual cases of OSCC [5]. The main cause of poor treatment outcome is local recurrence, even with microscopically negative surgical margins [7]. The clinical behavior therefore depends on the presence or absence of highly aggressive tumor cells. So, initial diagnosis with maximum accuracy is the key of successful treatment.

Cannibalism, the word, originated from the Spanish word *Cannibal* in connection with alleged cannibalism among the Caribs. This is also called anthropophagy (Greek *anthropos* means man and *pahagein* is to consume), which is the practice of humans consuming other humans. In zoology, Cannibalism is defined as any species consuming members of its own kind. Cellular cannibalism is not a new account in pathology; however, the significance of its presence has remained obscure until recently [19]. In 1904, Leyden first illustrated cellular cannibalism and he called them 'bird-eye cells' due to their morphological appearance under the microscope [13,19]. The phenomenon of tumor cannibalism has been compared to behavior of the microorganism, owing to its virulent nature of eating adjacent cells for their survival [19].

The tumor microenvironment plays an important role in the formation of cannibalistic cells [19]. These cells are particularly resistant to low pH and are formed in carcinogenesis in order to sustain or progress in unfavorable conditions such as low nutrient supply, hypoxia or starvation or as a tumor immune escape mechanism.A molecular framework of factors which contribute to the formation of cannibalistic cells include the presence of an acidic environment that allows continuous activation of specific lytic enzymes such as cathepsin B, caveolin formation and the actin linker molecule ezrin [13,19].Each of these molecular factors involved in tumor cannibalism may be new possible targets in future anti-tumor therapies [19].

Cellular cannibalism is fundamentally different from other forms of cell eating, such as phagocytosis, entosis, emperipolesis and autophagy, but can imitate this phenomenon's [20]. Therefore, for identification of cannibalistic cells, proper elucidation skills are essential. The morphology of the cannibalistic cell is composed of a crescent shaped nucleus engulfing another cell with a round to oval faded nucleus and this phenomenon was also noted during evaluation of CC (Figure 1).Brouwer et al. [21] proposed successive steps in the process of cannibalism with the initial process start with the

attachment of cannibalistic cell to a free cell pursued by gradual engulfment of the cell cytoplasm of the free cell, with alteration of the nucleus of the cannibalistic cell to semilunar shape; however, the nucleus of the free cell remains unaltered. Eventually the free cell gets completely interiorized within the cannibalistic cell and finally dies off [21]. Hence, we conducted a preliminary work to note the true significance of the presence of these cells.

On correlating CC grade with TNM staging and tumor size, we observed that a maximum of grade III (> 16) were in T4 tumor size and stage 4 of clinical TNM staging. It has been postulated that large tumor size at clinical presentation is often coupled with increased risk of local recurrence [22] and poor survival [23]. Clinical TNM staging and histopathology grading decides the treatment modalities, as they are the reflection of type of growth and degree of differentiation of the tumor.Accurate prognostification is often not reliable only with these parameters, but can improvise them by adding some more parameters [24].The present research suggests that, as the tumor size and stage increases, there is more propensity to have an increased number of cannibalistic cells adding to the aggressive nature of tumor cells.

Several authors have emphasized that number of cannibalistic cells can be correlated with the aggressive and metastatic behavior in systemic malignancies [14–18]. Moreover, Towers and Melamed have stated that increased number of cannibalistic cell may be useful in grading of breast carcinoma 25. OSCC consists of a diverse cell population with probable differences in invasiveness and metastasis behavior [7].Hence, clinical behavior depends on whether a tumor consists of highly aggressive cells or not.On correlating with histological tumor grade, the present results show that higher CC grade is not dependent exclusively on advanced histological grade. Our result was in disagreement with Sarode et al. [13], who reported an increased frequency of CC cells with higher histological grade.Moreover in their research they have highlighted more about the bizarre pattern of cannibalism, termed 'complex cannibalism'. In our present research we encountered very few suspicious cells showing such bizarre pattern of cannibalism.

Another major determinant of the prognosis of OSCC is the presence and extent of lymphatic invasion [26], which is pursued by increased probability for metastatic growth [27]. Up until now, no single dependable marker is available to predict the lymphatic metastasis of OSCC. In the present research all the positive lymph node cases were found to have higher grades of CC, i.e. grade III. Statistical analysis with Fischer exact test also showed a significant positive correlation. Lugini et al. [15] have also proven that CC not only eat adjacent tumor cells but also the defense cells of the immune response and suggested that this is a property of metastatic tumor cells.Therefore, the metastatic tumor cells may use cannibalism as a mechanism of tumor immune escape. Thus, the higher number of CC, the higher propensity for metastasis of tumor cells and this can be used as a useful prognosticator.

Follow-up history and CC grade did not show any significant correlation. On the other hand, those cases with documented recurrence and metastasis history had a higher CC grade reflecting the aggressive nature of OSCC. Although the number of cases were limited due to availability of only these with neck dissection and follow-up data in our archives. The true significance of our research outcome will be justified with larger sample size in the future scope of our study.

Conclusion

The outcome of the present research has revealed that CC grade has a significant association with lymph node metastasis of OSCC. Hence,cannibalistic cells can be considered as a prognostic marker for the aggressive nature of oral squamous cell carcinoma. We would like to propose that evaluation of cannibalistic cells should be done during routine histopathological assessment. Further research with a larger sample size and with a longer period of follow-up is required to establish the reliability of cellular cannibalism as an important and valid prognosticator in OSCC.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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