



Assessment of Tumor Cell Cannibalism as a Predictor of Oral Squamous Cell Carcinoma – A Histopathologic Correlation

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Abstract

Aim: Cellular cannibalism is defined as the ability of a cell to engulf another cell of its own type or any other. It has been recognized in various malignancies and is linked well with the aggressiveness, degree of anaplasia, invasiveness and metastatic potential. Literature search fetched up very few studies related to the presence and significance of cannibalism with respect to Oral squamous cell carcinoma (OSCC). The present study was aimed to detect tumor cell cannibalism in OSCC and to validate its role as a prognosticator of OSCC in relation to metastasis and degree of differentiation.

Method: 30 histopathologically proven cases, 15 cases each of metastatic OSCC (7 well differentiated OSCC and 8 moderately differentiated OSCC) and non-metastatic OSCC (8 well differentiated OSCC and 7 moderately differentiated OSCC) were included in the study.

Quantitative assessment of tumor cell cannibalism was done. The data was analyzed using Mann Whitney test.

Result: Metastatic OSCC showed higher frequency of cannibalistic cells compared to non-metastatic OSCC. More number of cannibalistic cells were found in moderately differentiated OSCC than well differentiated OSCC in both groups. Moreover, Grade III cannibalism and complex cannibalism was also found to be associated with metastatic, moderately differentiated OSCC exclusively.

Conclusion: It has been found that higher number of cannibalistic cells were associated with OSCC showing metastasis indicating their aggressive behavior. So, we recommend that quantitative assessment of tumor cell cannibalism should become a part of the routine histopathological examination of OSCC to screen its hostile behavior.

Keywords: Oral squamous cell carcinoma, Cannibalism, Metastasis, Histopathology

Introduction

The word “cannibalism” is derived from Spanish “caníbal” in connection with alleged cannibalism among Caribs. In Greek, it is known as anthropophagy, which refers to custom or act of humans eating other humans.^(1,2) Cellular cannibalism (CC) is defined as ‘a cell that is contained within another bigger cell with a crescent-shaped nucleus’. The fact behind this phenomenon is that the engulfed cell is enclosed in a big vacuole that pushes the nucleus of the cannibal cell to the periphery of the cell.⁽¹⁾ CC fundamentally differs from phagocytosis, entosis, emperipolesis and autophagy, though it may imitate this phenomenon’s.⁽²⁾

Exact mechanism by which tumor cells end up feeding on their sibling tumor cells remains ambiguous. However, recent literature suggests that tumor cell cannibalism

is a favorable event in malignancy and is thought to be accountable for tumor resistance against specific immune response. Also, tumor cells may use this process as a source of nourishment in setting of deficient nutrient supply and unreceptive tumor microenvironment.^(1, 2, 3) Another school of thought is that CC may function as a way to destroy tumor cells thus keeping a check on tumor growth.⁽²⁾

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CC is one of the distinctive and easily identifiable morphological trait often observed in aggressive malignancies and sometimes in benign tumors. ⁽³⁾ It has been observed cytologically as well as histopathologically in various malignancies like breast carcinoma ⁽⁴⁾, malignant melanoma ⁽⁵⁾, OSCC ^(6–8), endometrial stromal sarcoma ⁽⁹⁾, gastric adenocarcinoma ⁽¹⁰⁾ gastric micropapillary carcinomas ⁽¹¹⁾, metastatic adenocarcinomas ⁽¹²⁾, urothelial carcinoma ⁽¹³⁾, giant cell carcinoma of lung ⁽¹⁴⁾. These tumor cells ingest their siblings as well as other cells from the immune system in order to survive in hostile conditions of tumor microenvironment such as hypoxia, lack of nutrition and acidity. ⁽³⁾

Literature search fetched up few studies assessing this parameter as a prognosticator of Oral squamous cell carcinoma. This retrospective research was aimed to evaluate presence of tumor cell cannibalism in OSCC and to find its correlation with metastasis and grades of differentiation of OSCC.

Materials and Methods

Following approval of the research protocol from research advisory committee (2015/004/RAC/19) and institutional ethical review board (2015/400/IEC/19), 30 histopathologically proven, radical neck dissection cases, 15 cases each of metastatic and non–metastatic OSCC were included in the study. Only well differentiated and moderately differentiated grades of OSCC were considered (Table 1). We have not included poorly differentiated OSCC due to scarce availability and therefore to avoid sampling bias.

Identification of Tumor cell cannibalism: A large tumor cell enclosing or engulfing a slightly smaller cell in such a way that smaller tumor cells are lying within the larger tumor cells with sickle–shaped nuclei. Sarode et al (8) suggested the term “complex cannibalism” where one malignant cell was engulfing the other one and this complex was further engulfed by another cell.

Exclusion criteria: Overlapped tumor cells, dyskeratotic cells, vacuolated cells and tumors cells engulfing inflammatory cells have been excluded.

Counting of Tumor cell cannibalism and acquiring digital images: Each specimen was viewed under high power

Study Groups	Number of Cases	
	Metastatic OSCC	WDSCC=07
Non-Metastatic OSCC	WDSCC=08	MDSCC=07

Table 1. Distribution of study samples

(100X, oil immersion) using Olympus CX41 trinocular light microscope for assessing and counting of cannibalistic cells. The counting was done in 10 consecutive high power fields (hpf) and recorded as CC/10hpf. Figure 1a and 1b show tumor cell cannibalism in OSCC while Figure 2 shows complex cannibalism in OSCC.

Grading of Cannibalistic cells: Grade I: < 5 cells, Grade II: 6–15 cells, Grade III: >16 cells

Statistical analysis: Data collected was transferred to Microsoft Excel sheet followed by statistical analysis applying Mann Whitney using SPSS software (version 20). A value of $p < 0.05$ was considered statistically significant.

Results

Assessment of Tumor cell cannibalism in each study group:

- Higher mean cannibalistic cell counts were found in metastatic OSCC compared to non–metastatic OSCC and difference was found to be highly statistically significant (Table 2).

Groups	Mean	Standard Deviation	U-Value	p-Value	Result
Metastatic OSCC (N=15)	9.33	3.109	16.000	0.001	Highly Significant
Non-Metastatic OSCC (N=15)	5.53	1.407			

Table 2. Comparison of cannibalistic cell counts between Metastatic and Non–metastatic OSCC.

- Among metastatic group, MDSCC had significantly higher number of mean cannibalistic cell counts compared to WDSCC (Table 3).

- Similarly, in case of non–metastatic group, MDSCC exhibited significantly higher number of cannibalistic cells compared to WDSCC (Table 4).

- Among overall WDSCC and MDSCC, metastatic group showed higher mean cannibalistic cell counts compared to non–metastatic group and the statistical difference was found to be highly significant (Table 5 and Table 6).

Groups	Mean	Standard Deviation	U-Value	p-Value	Result
Metastatic WDSCC (n=7)	7.57	0.976	6.000	0.009	Significant
Metastatic MDSCC (n=8)	10.88	3.56			

Table 3. Comparison of cannibalistic cell counts between Metastatic WDSCC and Metastatic MDSCC.

Groups	Mean	Standard Deviation	U-Value	p-Value	Result
Non-Metastatic WDSCC (N=8)	4.75	0.886	9.000	0.023	Significant
Non-Metastatic MDSCC (N=7)	6.43	1.397			

Table 4. Comparison of cannibalistic cell counts among Non-metastatic WDSCC and Non-metastatic MDSCC

Groups	Mean	Standard Deviation	U-Value	p-Value	Result
Metastatic WDSCC (n=7)	7.57	0.976	1.000	0.001	Highly Significant
Non-Metastatic WDSCC (n=8)	4.75	0.886			

Table 5. Comparison of cannibalistic cell counts among Metastatic WDSCC and Non-metastatic WDSCC

Discussion

Cellular cannibalism (CC) is known as the ability of a cell to engulf another cell of its own type or any other cell, either homogenous or heterogeneous type.⁽¹⁵⁾ CC in cytological smears or in histopathology is manifested as a cell that is within a larger cell having a crescent shaped nucleus. This characteristic appearance is ascribed to the fact that ingested cell is enclosed in a large vacuole that shoves the nucleus of cannibalistic cell to the boundary.⁽³⁾ Leyden in 1904 described this appearance as “bird-eye cells”.⁽¹⁶⁾ The process of cannibalism occurs in successive steps as described by Brouwer et al.⁽¹⁷⁾ CC varies from other types of cell in cell phenomena like phagocytosis,

Groups	Mean	Standard Deviation	U-Value	p-Value	Result
Metastatic MDSCC (n=8)	10.88	3.563	2.000	0.002	Highly Significant
Non-Metastatic MDSCC (n=7)	6.43	1.397			

Table 6. Comparison of cannibalistic cell counts among Metastatic MDSCC and Non-metastatic MDSCC.

emperipolesis, entosis and autophagy although, it can emulate these process.⁽³⁾

It is seen that during carcinogenesis most cancer cells displace their metabolic pathway from the metabolism showing Pasteur-effect into the one exhibiting Warburg-effect which in turn results in an acidic microenvironment and ultimately creating a positive carcinogenesis feedback loop. In response to this acidic condition, the tumor surroundings facilitate sorting of some virulent cells that are capable to thrive in such hostile conditions at the cost of other cells. Thus, tumor microenvironment is an imperative facet that further governs this process. So, unfavorable circumstances such as hypoxia, lack of nutrition and acidic condition favors cannibalism.⁽¹⁸⁾

CC is well recognized histopathological parameter and has been reported as an indicator of aggressiveness, anaplasia and metastatic potential in several malignancies.^(4, 6, 7, 12) Available literature showed few studies which have utilized this parameter as a prognosticator of OSCC.^(6, 7, 8) The present study was intended to assess tumor cell cannibalism in metastatic and non-metastatic OSCC as well as to find its correlation with degree of differentiation. The findings of our study showed significantly higher

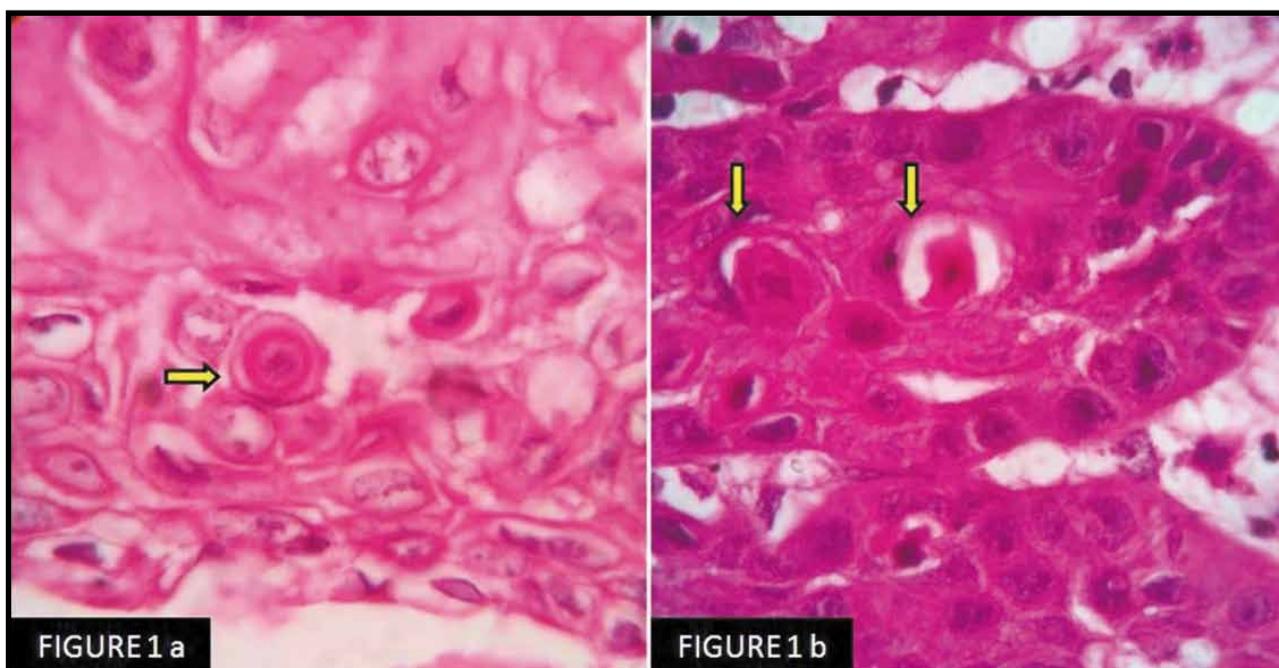


Figure 1a and 1b. Tumor cell cannibalism in OSCC. (H & E stain, Mag-100x, oil immersion)



Figure 2. Complex cannibalism in OSCC. (H & E stain, Mag–100x, oil immersion)

number of cannibalistic cells in metastatic group compared to non–metastatic. Also, Grade III cannibalism and complex cannibalism was also found to be associated with metastatic OSCC solely. This finding is in accordance with the study done by Jose et al ⁽⁷⁾ and suggest that tumor cell cannibalism is an unfavorable histopathological prognostic factor.

When we compared the cannibalistic cell counts within metastatic group, it was found to be significantly higher in MDSCC than WDSCC. Likewise, within non–metastatic group, MDSCC showed significantly higher number of cannibalistic cells compared to WDSCC. When overall WDSCC and MDSCC were compared, metastatic group showed higher mean cannibalistic cell counts compared to non–metastatic group and the correlation was found to be highly statistically significant. We also observed that WDSCC cases demonstrated Grade I and Grade II cannibalism while none of them showed grade III type. On the other hand, MDSCC cases were associated with Grade II type and exclusively Grade III and complex cannibalism. Thus, we can conclude that tumor cell cannibalism is valid marker to assess aggressiveness. However, these finding are not in agreement with study by Jose et al ⁽⁷⁾ who did not find any significant correlation between cannibalism grade and histologic differentiation. Also, Sarode et al ⁽⁶⁾ found maximum cannibalistic cells in advanced grades of OSCC but the correlation was not statistically significant.

Conclusion

CC is an easily identifiable histopathological feature under light microscopy and is reported to be usually associated with aggressive malignancies with anaplastic morphology. It has been regarded as an adaptive strategy of the malignant cells for survival benefit. Our data extend this observation to OSCC, a tumor histotype typified by violent conduct and adverse outcome and to find its correlation with metastasis and degree of differentiation. Our result showed that tumor cell cannibalism has a vital role in estimating the biological behavior of OSCC. It is found to be significantly associated with lymph node metastasis and grades of differentiation. So, we recommend that quantitative assessment of tumor cell cannibalism should become a part of the routine histopathological diagnosis for OSCC.

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