International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 9; Issue 10 (B); October 2020; Page No. 23193-23196
DOI: http://dx.doi.org/10.24327/ijcar.2020.23196.4594

Research Article

ASSESSMENT OF CELLULAR CANNIBALISM IN PREDECTING THE AGGRESSIVE NATURE OF ORAL SQUAMOUS CELL CARCINOMA

Suwasini, Manisha Shrikaar and Madhuresh Kumar*

Department of Oral Pathology & Microbiology,
Buddha Institute of Dental Sciences & Hospital, Patna

A R T I C L E I N F O

Article History:
Received 10th July, 2020
Received in revised form 2nd August, 2020
Accepted 26th September, 2020
Published online 28th October, 2020

Key words:

Cannibalistic Cell (CC), Cellular Cannibalism, Oral Squamous Cell Carcinoma (OSCC).

A B S T R A C T

Background: Cellular cannibalism has been considered as an important morphologic feature exclusively seen in aggressive malignancies. It is defined as the ability of a cell to engulf another living cell leading eventually to the death of the internalized cell. It has been seen in several malignancies and they are associated with the degree of anaplasia, invasive and metastatic potential of tumor cells. Cannibalistic cells (CC) could serve as a valuable tool in assessing tumor behavior in oral squamous cell carcinoma (OSCC).
Aim: The aim of our study was to evaluate the presence of CC and to correlate its role in aggressive nature of OSCC.
Material and methods: 30 histopathologically proven cases of OSCC were included in our study. The CC was evaluated in the lesional tissue and the data was analyzed using Chi Square test.
Result: All the cases showed presence of CC. Grade 1 CC was found in 12 cases, Grade II CC was found in 9 cases, Grade III CC was found in 9 cases.
Conclusion: Cannibalistic cells can easily identifiable under light microscope without use of any advanced and expensive molecular techniques. Thus aggressive nature of OSCC can be routinely assessed.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm of oral cavity, which is usually preceded by potentially malignant disorder.[1] In spite of the various research related to the diagnostic modalities and management aspects of OSCC, the mortality rates are still low with 5-year survival rate being <50%.[2] Various histological markers have been shown to have prognostic impact[2] along with molecular studies that has enhanced the standard of cancer diagnosis; but still histopathology remains the gold standard.[3] Advanced diagnostic modalities have been proposed as prognosticators in carcinomas; but cannot be used on routine clinical practice as these facilities may not be possible or affordable for all the patients.[3] OSCC consists of a diverse cell group having different metastatic and invasive behavior, but there is a lack of relationship between grades and the prognosis of OSCC. Histological parameters like tumor thickness, lymphovascular invasion, microvascular density, perineural spread at the invasive front, and tissue eosinophilia have been described as major risk factors that adversely affect the prognosis of patients. 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. [3] One such important prognostic parameter that has a vital role in estimating the aggressive nature of OSCC is cellular cannibalism.

Cannibalism is the term used to describe "Cell-Eat-Cell" phenomenon.[4]The word cannibalism is derived from cannibals which is the Spanish name for the Carib people formerly well known for practicing cannibalism i.e practiced of humans eating the flesh or internal organs of other human beings.[3-8] Cellular cannibalism is known as the ability of a cell to engulf another cell of its own type or any other cell, either homogenous or heterogenous type.[6,9] The phenomenon of cell cannibalism has been observed in several tumors such as lung carcinoma, renal carcinoma, bladder carcinoma, breast carcinoma, endometrial stromal sarcoma, gastric adenocarcinomas, malignant melanoma, and lymphomas. [5,10] As it is easily identifiable and vital marker of aggressive biological behavior but often ignored during routine histopathological assessment of OSCC. Hence, the aim of this study was to evaluate the presence of cannibalistic cells and to correlate its role in aggressive nature of OSCC.

MATERIALS AND METHODS

A total number of 30 archival paraffin- embedded blocks obtained from the Department of Oral Pathology and Microbiology BIDSH. An Institutional Ethical Committee

Corresponding author:* **Madhuresh Kumar
Department of Oral Pathology & Microbiology, Buddha Institute of Dental Sciences & Hospital, Patna

approval (Letter No- 459/BIDSH) was obtained before the start of the study. Demographic data regarding age, gender, site, TNM stage of all cases were noted from the records. The sections were stained with H & E and analyzed further to confirm the diagnosis and were graded on the basis of Borders' system as well, moderate, poorly differentiated OSCC and later tumor cells were evaluated for the presence of cannibalistic cells (CCs).

Identification of tumor CC A larger tumor cell that engulf the another smaller tumor cells with a crescent shaped nucleus was considered as a CC.

Exclusion criteria Overlapped tumor cells, dyskeratotic cells, tumor cells engulfing inflammatory cells have been excluded during evaluating CC.

Counting of tumor cell cannibalism The sections were observed under light microscope (Multi viewing Microscope Model CXR5) with x 400 magnification at 10 different fields. Based on frequency of presence of CC, grade were given: CC Grade I: <5 cells, CC Grade II: 6-15 cells, CC Grade III: >16 cells.

To find the correlation between CC grade and biological behavior of tumor, a comparison of CC grade with clinical staging and histopathological grading were done.

Statistical procedures

Data obtained was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States). Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM). Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data has been depicted. Comparison of frequencies of categories of variables with groups was done using chi square test.

RESULTS

Demographic results

80% patients were below 65 years whereas 66.66% cases were male. Most patients were of clinical staging of stage IV (40%). Majority of cases were of WDSCC (50%). [Table: 1]

Demographic results	Number of patients	%
Age		
<65	18	60%
>65	12	40%
Gender		
Male	20	66.66%
Female	10	33.33%
TNM		
Stage II	8	26.66%
Stage III	10	33.33%
Stage IV	12	40%
Histopathology		
WDSCC	15	50%
MDSCC	10	33.33%
PDSCC	05	16.66%

Clinical staging and CC

Out of 30 cases 8 cases were of stage II, 10 cases were of stage III and 12 cases were of stage IV of clinical TNM. There was a statistically significant / highly significant difference seen for the frequencies between the groups (p= 0.000) with higher frequencies for CC Grade I with TNM Stage II & CC Grade III with TNM Stage IV. [Table: 2, Figure: 1]

Table 2:Table representing clinical staging and CC		TNM Stage			
		TNM Stage II	TNM Stage III	TNM Stage IV	Total
CC Grade	CC Grade I	08	04	00	12
	CC Grade II	00	04	05	09
	CC Grade III	00	02	07	09
	Total	08	10	12	30

Chi square value = 21.222, p = 0.000

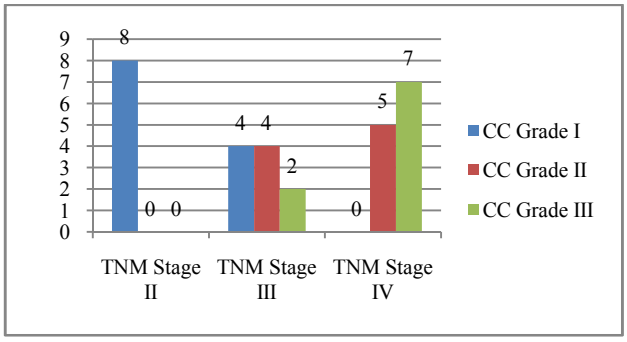

Figure 1 Bar graph showing TNM staging and CC grade

Histopathology grading & CC grade

Out of 30 cases 15 cases were diagnosed as WDSCC, 10 were MDSCC, 5 was PDSCC. CC was found in all cases (100%) of OSCC. Grade I CC was found in 12 cases (10 in WDSCC, 2 in MDSCC). Grade II CC was found in 9 cases (4 in WDSCC, 4 in MDSCC, 1 in PDSCC). Grade III CC was found in 9 cases (1 in WDSCC, 4 in MDSCC, 4 in PDSCC). There was a statistically significant / highly significant difference seen for the frequencies between the groups (p=0.009) with higher frequencies for CC Grade I with WDSCC & CC Grade III with MDSCC & PDSCC.[Table:3, Figure:2]

Table 3: Table representing histopathology and CC		Histopathological Grade			
		Well diff SCC	Mod diff SCC	Poorly diff SCC	Total
CC Grade	CC Grade I	10	02	00	12
	CC Grade II	04	04	01	09
	CC Grade III	01	04	04	09
	Total	15	10	05	30

Chi square value = 13.444, p = 0.009

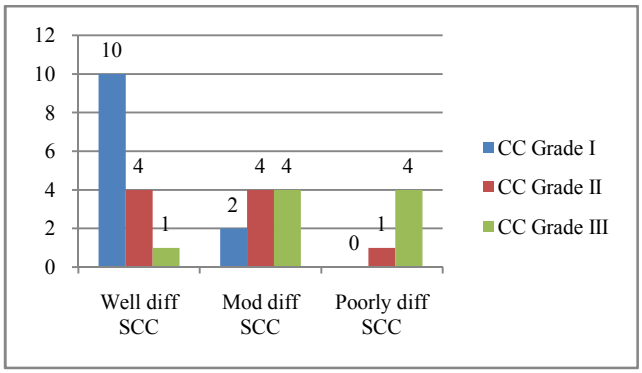

Figure 2 Bar graph showing Histopathology and CC grade

There was a statistically significant / highly significant difference seen for the frequencies between the groups (p=0.009) with higher frequencies for CC Grade I with WDSCC & CC Grade III with MDSCC & PDSCC

Total encirclement of the inner tumor cell by the host cell membrane, a semilunar host cell nucleus, and a round shape of the tumor cell was seen. [Figure: 3, 4, 5, 6]

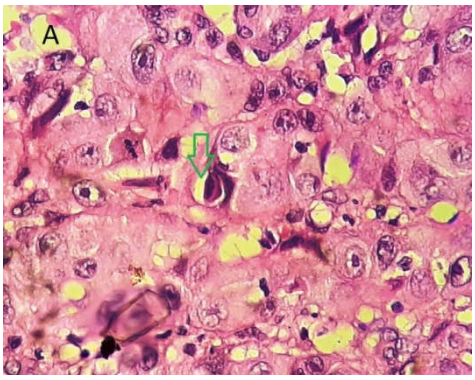

Figure 3

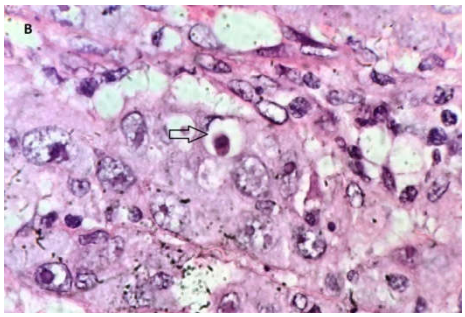

Figure 4

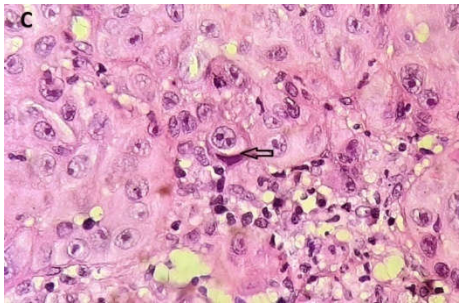

Figure 5

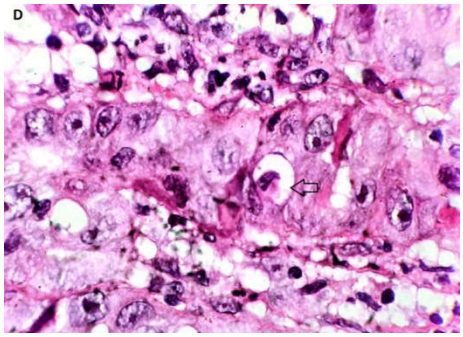

Figure 6

There was a statistically significant / highly significant difference seen for the frequencies between the groups (p= 0.000) with higher frequencies for CC Grade I with TNM Stage II & CC Grade III with TNM Stage IV.

DISCUSSION

Cannibalism was first described by Leyden in 1904, who called them "bird's-eye cells" due to their morphological appearance under the microscope.[3,11] Other terms used in the literature to describe this phenomenon are "cellular phagocytosis," "cell phagocytosis," "cell-in-cell appearance," "cell-in-cell pattern," "one cell delicately wrapped around the next," "tumor cell within a tumor cell," "phagocytosis of tumor cell by tumor cell" and "tumor cell embraced by another tumor cell."[10]

Cell engulfments can occur homotypically (between cells of the same type) or heterotypically (between cells of different types). Until recently, cannibalism was recognized as a phenomenon seen mainly with tumor cells ingesting other tumor cells but recent reports have shown that tumor cell engulfs other cells as well, such as neutrophils, lymphocytes, and erythrocytes. This is known as xeno-cannibalism. Thus, the term cell cannibalism is described as the ability of tumor cells to cannibalize their siblings' neoplastic cells as well as stromal or tumor infiltrating immune cells. [12]

CC in cytological or histological preparation is manifested as a cell that is contained within another bigger cell with a crescent shaped nucleus. This particular appearance is attributed to the fact that ingested cell is contained in a big vacuole that pushes the nucleus of cannibalistic cell to the periphery. [7] Apart from conventional cannibalism there is another form of cannibalism in which one malignant cell engulfs another, and this complex is further engulfed by another cell. Alternatively, one cell may engulf two cells at once. This is called "complex cannibalism."[7, 12, 13]

The mechanism of cannibalism has been ascribe to the metabolic alterations in the malignant tumor cells, that supports cannibalization of cells within other similar tumor cells, in adverse conditions of hypoxia, decreased nutritional state and acidic states. This leads to the selection of certain resistant cell phenotypes over others in caustic environment. These malignant cells are highly virulent and cannibalize other malignant cells and help them survive and progress in such difficult conditions. [10]

Tumor microenvironment also plays an important role in the formation of CCs. 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 CCs 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. Each of these molecular factors involved in tumor cannibalism may be new possible targets in future antitumor therapies.[3,4,8]

CC is one of the typical morphological traits often observed in aggressive malignancies, although it has been demonstrated in certain benign tumors also.[7] CC has easily identifiable morphological features under light microscopy without the use of any advanced and expensive molecular techniques. Hence, aggressiveness of the neoplasm can be assessed on a routine basis.[7]

Clinical TNM staging along with histopathological grade decides the treatment modalities, as they reflects the type of growth and degree of differentiation of the tumor. [3] Several authors focused that number of CC can be correlated with the aggressive nature of tumor. We found highly significant difference between the groups ($P<0.01$) with higher frequencies for CC Grade I with TNM Stage II and CC Grade III with TNM Stage IV. D. Jose et al correlated CC grade with TNM staging and tumor size, and observed that a maximum of

Grade III CC (> 16) were in T4 tumor size and stage 4 of clinical TNM staging. They postulated that larger tumor size at clinical presentation is often coupled with increased risk of local recurrence and poor survival.[3]

So, the present study reveals that as the clinical TNM staging increases there was more frequency of CCs that adds the aggressive nature of tumor cells.

In our study we found statistically highly significant difference between the groups (P<0.01) with higher frequency of CCs grade I with WDSCC and CC Grade III with MDSCC and PDSCC. So it can be concluded that increased number of CCs can be related to the poor prognosis. Sarode et al found that PDSCC had more number of cannibalistic cells per high power field as compared to MDSCC but the correlation was not statistically significant. [14]

Megha et al. assessed tumor CC in metastatic and non-metastatic OSCC as well as found its correlation with degree of differentiation where they found significantly higher number of cannibalistic cells in metastatic group compared to non- metastatic group. They also observed that WDSCC cases demonstrated Grade I and Grade II CC while none of them showed Grade III type, whereas MDSCC cases were associated with Grade II type and exclusively Grade III and complex cannibalism.[6]

Limitations

Our study was performed involving only 30 sample size, which accounts our biggest limitation. Hence, our study provides a template for future researches with a larger sample size to be conducted to achieve a more reliable and authentic data.

CONCLUSION

Cannibalistic cells are important morphological parameters that can easily identifiable in routine histopathology and can be easily assessed without use of any advanced and expensive molecular techniques. It is correlated well with aggressiveness, degree of anaplasia, invasiveness and metastatic potential. Demonstration of an increased number of CCs may also be a parameter in grading OSCC. Our present study revealed that cannibalistic cells can be used to determine the aggressive nature of OSCC. However larger sample sizes are needed to validate these findings.

References

1. Sarode GS. Efferocytosis in oral squamous cell carcinoma. J Oral Maxillofac Pathol 2016;20:170-2.
2. Kale AD, Angadi PV. Tumor budding is a potential histopathological marker in the prognosis of oral squamous cell carcinoma: Current status and future prospects. J Oral Maxillofac Pathol 2019;23:318-23.
3. Jose D, Mane DR, Datar U, Muttagi S, Hallikerimath S, Kale AD. Evaluation of cannibalistic cells: A novel entity in prediction of aggressive nature of oral squamous cell carcinoma. Acta Odontol Scand 2014;72:418-23.
4. Kale A. Cellular Cannibalism. *J Oral Maxillofac Pathol* 2015;19:7-9.
5. Kala S, Kaur G. Cellular cannibalism: A promising feature to determine cancer prognosis. J Oral Res Rev 2016;8:45-7.
6. Jain M, Saawarn S, Gupta A, Ashok S, Mhaske S, Khan S, Jain M. Assessment of Tumor Cell Cannibalism as a Predictor of Oral Squamous Cell Carcinoma- A Histopathologic Correlation. G.J.O.Issue 23,2017
7. Jain M. An Overview On "Cellular Cannibalism" With Special Reference To Oral Squamous Cell Carcinoma. Exp Oncol 2015 37, 4, 242–245
8. Fais S. Cannibalism: A way to feed on metastatic tumors. Cancer Letters 258 (2007) 155–164.
9. Krajcovic M Overholtzer M. Mechanisms of ploidy increase in human cancers: a new role for cell cannibalism. Cancer Res. 2012 April 1; 72(7): 1596–1601.
10. Gupta N, Jadhav K, Shah V. Emperipolesis, entosis and cell cannibalism: Demystifying the cloud. *J Oral Maxillofac Pathol* 2017; 21:92-8.
11. Bansal C, Tiwari V, Singh US, Srivastava AN, Misra JS. Cell Cannibalism: A cytological study in effusion samples. *J Cytol* 2011; 28:57-60.
12. Siddiqui S, Singh A, Faizi N, Khalid A. Cell cannibalism in oral cancer: A sign of aggressiveness, de-evolution, and retroversion of multicellularity. *J Can Res Ther* 2019; 15:631-7.
13. Wang X, Li Y, Li J, Li L, Zhu H, Chen H, Kong R, Wang G, Wang Y, Sun B. Cell-in cell Phenomenon and its relationship with tumor microenvironment and tumor progression: a review. Frontiers in cell and developmental biology. 2019.Vol 7;article 311
14. Sarode SC, Sarode GS. Identification of cell cannibalism in oral squamous cell carcinoma with clinic-pathological correlation. Oral Oncol 2013; 49: S90-1.

How to cite this article:

Suwasini, Manisha Shrikaar and Madhuresh Kumar (2020) 'Assessment of Cellular Cannibalism in Predecting the Aggressive Nature of Oral Squamous Cell Carcinoma', *International Journal of Current Advanced Research,* 09(10), pp. 23193-23196. DOI: http://dx.doi.org/10.24327/ijcar.2020.23196.4594

****