Original Article

A Correlation of Serum Histamine and Mast Cell Count with the Established Prognosticators in Oral Cancer

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Abstract

Context: We undertook this study in view of the rising interest in the interactions between tumor cells and mast cells in the tumor microenvironment and the mounting evidence of the role of mast cells in the prognosis of various cancers. **Aims:** The aim of the study is to determine the prognostic utility of mast cells and their mediators in oral squamous cell carcinoma (OSCC). **Settings and Design:** This is a cross-sectional analytical study. A total of 41 cases were studied for determining the correlation of mast cell counts with histopathological prognostic variables and 29 cases for determining the serum histamine levels and to further correlate the values with the histopathologic determinants. **Subjects and Methods:** Mast cell assessment was done using Toluidine blue stain on tissue sections while histopathological prognostic factors were evaluated with routine stains. The serum histamine levels were obtained by sandwich enzyme linked immunosorbent assay (ELISA). **Statistical Analysis:** Mann–Whitney *U*-test was used to test the difference between parameters and a Spearman correlation coefficient was used. $P < 0.05$ was considered significant for statistical analysis. **Results:** We found statistically significant correlations between increased median mast cell counts and higher grade of tumor, presence of lymphovascular invasion, greater depth of invasion, and presence of regional lymph node involvement. The serum histamine values correlated only with the depth of tumor invasion. **Conclusions:** Hence, as per our results, we suggest the use of mast cell counts in OSCC cases as a prognostic indicator in conjunction with other clinicopathological factors. At the same time, we acknowledge the fact that extensive studies are required to establish the role of mast cell mediators in the prognostic curve of oral cancer patients.

Keywords: ELISA, histamine, mast cells, oral cancer, toluidine blue

Introduction

In India, oral cancer is the third most common cancer and accounts for 30% of all cancers.[1] Oral squamous cell carcinomas (OSCC) account for 95% of all oral malignancies.[2] The average incidence is estimated to be around 20 per 100,000 people.[3] The regional variations in the incidence may result from the variations in the prevalence of risk factors.[4] Several risk factors have been implicated in the development of oral cancer of which tobacco abuse and betel quid chewing are the established factors.[5] In spite of recent advances in diagnostic and therapeutic techniques, the disease course of these cancer patients continues to be dismal. For these reasons, an adequate armament of prognostic indicators is required to personalize the treatment as much as possible.

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Although there have been recent studies on the role of mast cells in tumors, conclusive evidence on the exact impact of mast cells in the tumor microenvironment remains elusive. Many authors have explored the significance of tumor-associated mast cells in cancers such as prostate,[6] breast,[7] lung,[8] esophagus,[9] and stomach.[10] Few studies conducted on mast cells in oral cancers have focused mainly on the molecular epidemiology of mast cell populations and their role in angiogenesis.[11] There has been compelling evidence of increased mast cell density in OSCC when compared with the normal and dysplastic oral mucosa.[12] However, the exact role (pro-tumorigenic versus antitumorigenic effect) of mast cells and their mediators in

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cancers is inconclusive to date. Recent research indicates a potential role of histamine in oral carcinogenesis.[13]

The aim of this study is to correlate the mast cell density and serum level of mast cell mediator like histamine with the various pathological prognostic indices of OSCC. It is also an attempt to find the prognostic utility of mast cells and its mediators in OSCC.

Subjects and Methods

Study design

This is a cross-sectional analytical study where all the study subjects are cases of OSCC. It includes two sets of cases. The mast cell count was obtained from 41 cases while the serum histamine values were obtained from another 29 cases of oral cancer.

Period and place of study

The study was conducted in the Department of Pathology of a tertiary care hospital equipped with all super specialty units. It was done from June 2019 to July 2019 for a period of 2 months.

Inclusion criteria

1. All cases have been included after obtaining informed consent from the patients
2. For determining the mast cell counts: Cases of OSCC involving lips, anterior tongue, floor of mouth, buccal mucosa, upper and lower alveolar ridges, hard palate, and retromolar trigone which had been diagnosed and treated by radical surgical procedures. These were obtained from the archival histopathological records of May 2018 to May 2019
3. For obtaining the serum histamine values, cases of suspected oral cancer that underwent biopsy procedures and were given a diagnosis of squamous cell carcinoma within the study period of 2 months (June, July 2019) before any neoadjuvant chemotherapy or surgery was done.

Exclusion criteria

For total mast cell count

a. Oral malignancies with a histopathologic diagnosis other than squamous cell carcinoma
b. Patients who had received prior treatment (neoadjuvant chemotherapy) for cancer.

For serum histamine determination

a. Cases of OSCC who had undergone any form of treatment like radiotherapy or surgery
b. Patients of oral SCC with a history of allergic reactions or any other systemic disease
c. Patients of oral SCC with bleeding diathesis.

The clinicodemographic data were collected first, including age, gender, socioeconomic status, personal habits, and presence of any comorbidities. Mast cell counts were performed on the tissue sections of radically resected cancer specimens which were received in the histopathology section of the Department of Pathology. The specimens were kept for fixation with 10% neutral buffered formalin for 24 h, following which the specimens were grossed and appropriate representative tissues were sampled for histopathological assessment after noting down the gross tumor dimensions and distance from the margins. The stained slides of the cases selected from the archival were retrieved. Histopathological assessment was done for various parameters such as depth of tumor invasion, grading of tumor, staging, presence of lymphovascular and perineural invasion. The tumors were graded according to Broder's system which includes well-, moderately-and poorly-differentiated squamous cell carcinomas. The 8th edition American Joint Committee on Cancer tumor, lymphnode involvement, metastasis (TNM) staging was used which includes the size of tumor, its extent of local extension, nodal involvement, and distant metastases.

For mast cell assessment, first, the representative tumor sections were chosen and an unstained slide was prepared from the archival paraffin blocks. Only completely excised primary neoplasms containing adequate amounts of connective tissue beyond the tumor–host interface were included. Then the slides were stained using the metachromatic stain, 1% Toluidine blue HiMedia laboratories (HIMEDIA). The toluidine blue stains the granules of mast cells purplish–red and the background blue. First, the slides were scanned in low magnification for detecting the areas with maximum positively stained mast cells (hotspots). Then, the counting was done in high power (×400) magnification. Ten consecutive high-power fields in the hotspots were counted to obtain the total mast cell count per ten high power fields (HPF). Care was taken not to overlap the fields during the counting process. The counts obtained were then evaluated for any significant associations with depth of tumor invasion, grading of tumor, staging (TNM), lymphovascular invasion, and perineural invasion.

The serum histamine levels were obtained using a sandwich ELISA kit (BT® Laboratory) with the help of an ELISA Analyser (EuroImmuno® Chemwell 2910). A total of 0.2 ml of a patient's peripheral blood sample was collected from the left arm in an Ethylenediaminetetraacetic acid tube, shaken well and immediately transported to the lab. First, it was centrifuged at 1000 rpm for 8 min, following which it was stored. Upon collection of a significant number of samples, the serum of the patients was placed in a micro-ELISA plate, in which 20 µl of antibody solution was added. After adding the chromogenic substance, the samples were analyzed. The serum histamine levels were then obtained from the readings taken and tabulated.

Data entry was done in MS®Excel and analyzed by SPSS® 25.0 (IBM Inc., IBM Corp, Armonk, NY). Categorical variables were expressed in terms of percentage/proportions, while continuous variables like mast cell density and serum histamine levels were presented as mean (standard deviation) or median interquartile range. The differences in mast cell counts and serum histamine levels across prognostic factors were tested using Mann–Whitney *U*-test. The correlations between mast cell count and depth of tumor invasion were

determined by Spearman coefficient with $P < 0.05$ considered to be statistically significant.

Ethical clearance was obtained from the Institutional Ethical Committee, and informed consent was obtained from the participants of the study. IRB Board name – Institutional Ethical Committee, Institute of Medical sciences and SUM Hospital, S 'O'A University, Approval Number – IMS.SH – SOA- 180226, Approval Date - 7th June 2019. Informed consent was obtained in compliance to the Declaration of Helsinki.`

Results

Taking into account all the study participants, the median age of presentation of OSCC in this study is 47 years. Of all the sites that were involved in this tumor, the most common affected site was the tongue ($n = 24$ cases) [Figure 1]. Males ($n = 59$ cases) were more commonly affected than females ($n = 11$ cases) [Table 1]. All the cases which were included in the study were diagnosed as squamous cell carcinoma after histopathological examination of the biopsy samples. The tumors were graded as per Broder's classification as well-, moderately-, and poorly differentiated. The majority (48.6%) of tumors were classified as moderately-differentiated squamous cell carcinomas followed by well-differentiated tumors (47.1%) [Table 1].

Depth of tumor invasion is an important prognostic variable and is defined as the distance from the basement membrane of the adjacent uninvolved mucosa until the maximum extent of tumor invasion into stroma. The depth of invasion of 80% of the tumors was <10 mm, while 44.3% of the squamous cell carcinomas had lymphovascular invasion of the tumor cells and 32.9% cases showed perineural invasion [Table 1]. Only 13 cases (18%) showed metastases to regional lymph nodes.

Table 1: Distribution of demographic and pathological characteristics ($n = 70$)

Variable	Characteristics	Number of cases, *n* (%)
Sex	Female	11 (15.7)
	Male	59 (84.3)
Tumor grade	G1	33 (47.1)
	G2	34 (48.6)
	G3	3 (4.3)
Depth of invasion (mm)	<10	56 (80)
	>10	14 (20)
PNI	No	47 (67.1)
	Yes	23 (32.9)
LVI	No	39 (55.7)
	Yes	31 (44.3)
T*	1	8 (19.5)
	2	21 (51.2)
	3	11 (26.8)
	4	1 (2.5)
N*	0	27 (65.9)
	1	8 (19.5)
	2	5 (12.2)
	9	1 (2.4)

*Stands for 41 (cases from archival records). PNI: Perineural invasion, LVI: Lymphovascular invasion, T: Tumor, N: Lymphnodes

The mast cells were recognized by their purplish–red metachromatic granules present in the cytoplasm. These cells were counted in the peritumoral hotspots and were expressed per ten HPF [Figure 2]. The median mast cell count in low-grade (well-differentiated) tumors was 36 whereas in higher grade tumors (moderately and poorly differentiated), it was 46. This difference was statistically significant ($P < 0.001$) [Table 2]. An increase in the median mast cell counts was associated with increase in depth of tumor invasion ($P = 0.05$) [Figure 3], presence of lymphovascular invasion (<0.001) [Table 3], and regional lymph node involvement ($P < 0.001$) [Table 4]. Although a higher median mast cell count was observed in tumors with the presence of perineural invasion, this difference was not statistically significant [$P < 0.08$ [Table 5]].

The serum histamine levels were expressed in ng/ml. A higher serum histamine level was seen in lower grade tumors compared to higher grade tumors, but this difference was not statistically significant ($P = 0.58$) [Table 6]. Although higher serum histamine levels were observed in tumors with evidence of lymphovascular and perineural invasion, this difference was not statistically significant [Tables 7 and 8]. With increase in depth of invasion, there was a significant increase in serum histamine levels ($P = 0.059$) [Table 9].

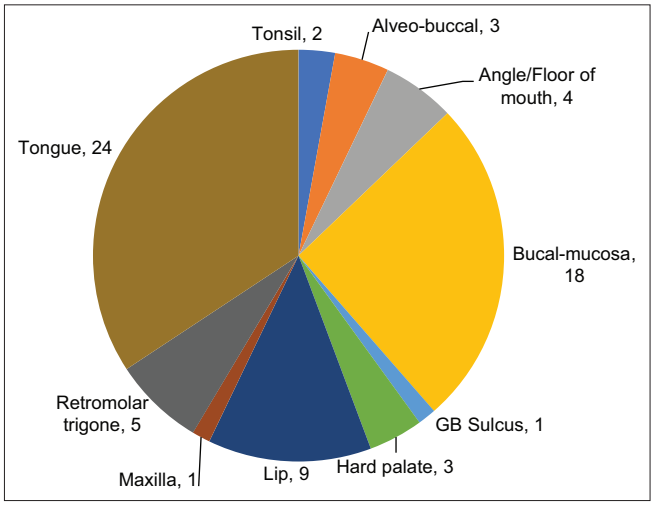

Figure 1: Location of the tumour ($n = 70$)

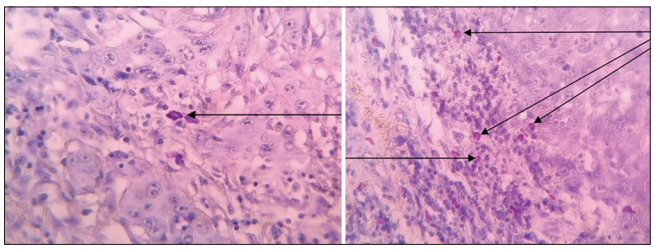

Figure 2: Mast cells in the peritumoral area highlighted by the Toluidine blue stain (Arrow marks show metachromatic granules stained in mast cells)

Discussion

The presence of mast cells in the peritumoral areas was reported for the first time by Westphal, following which a substantial amount of evidence has been generated regarding tumor-associated mast cells in various cancers of the human body. Mast cells are well known for the plethora of mediators secreted by them which have been suggested to aid in vital steps of carcinogenesis. This study was undertaken to explore the role of mast cells and their mediators in oral cancers.

The present study showed a positive correlation of mast cell count with histological grades. Increased mast cell counts were observed in higher tumor grades ($P < 0.001$). This was in concordance with previous studies, such as Sharma *et al*. who noted significantly higher mast cell densities in moderately-differentiated squamous cell carcinomas as compared to well-differentiated carcinomas ($P < 0.001$).[14] On the contrary, a study by Cheema *et al*. concluded that mast cell density was significantly higher in well-differentiated squamous cell carcinoma than in other histological grades.[15] However, certain studies did not find any correlation of mast cell densities with the various grades of squamous cell carcinoma.[16,17] The possible explanations for such conflicting results have been suggested by Tomita *et al*.[18] The cytotoxic functions of mast cells may be initially present, but following tumor stromal microenvironment interaction, the cytotoxic functions might be suppressed, leading to tumor progression. Second, the impact of mast cells on the tumor might have a dependence on the concentration of mediators released by the mast cells in the tumor microenvironment.

There was also a positive correlation between the mast cell counts and depth of invasion ($P < 0.05$), lymphovascular invasion ($P < 0.001$) and regional lymph node involvement ($P < 0.001$) [Table 4] in our study. However, no significant association was seen between mast cell counts and status of perineural invasion in the tumors ($P = 0.08$). A similar finding was observed by Alaeddini *et al*.[19] In their study, mast cell density did not show significant relationship with a histological risk assessment model, which included the worst pattern of invasion, perineural invasion, and lymphocytic infiltration.

Mast cell mediators such as histamine, heparin, and fibroblast-derived growth factor have been suggested as the

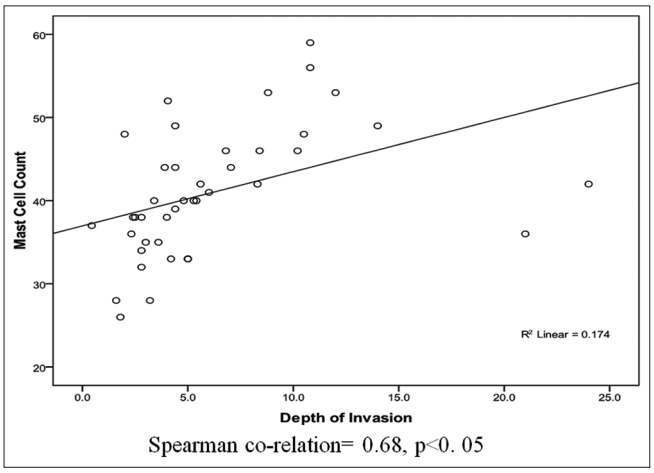

Figure 3: Co-relation between Mast cell count and Depth of invasion (n=41)

Table 2: Mast cell count across grades of tumor

Grade of tumor	*n*	Mean	SD	Median mast cell count/10 HPF
Low	18	35.39	4.680	36.00
High	23	45.39	6.652	46.00
Total	41	41.00	7.675	40.00

P<0.001 (Mann–Whitney U-test) (Low versus high). SD: Standard deviation, HPE: High-power fields

Table 3: Mast cell count across lymphovascular invasion

LVI	*n*	Mean	SD	Median mast cell count/10 HPF
Absent	28	37.54	5.634	38.00
Present	13	48.46	6.050	49.00
Total	41	41.00	7.675	40.00

P<0.001 (LVI absent versus present). SD: Standard deviation, LVI: Lymphovascular invasion, HPF: High-power fields

Table 4: Mast cell count across nodal involvement status

Nodal involvement	*n*	Mean	SD	Median mast cell count/10 HPF
Absent	28	37.86	5.986	38.00
Present	13	47.77	6.559	48.00
Total	41	41.00	7.675	40.00

P<0.001. SD: Standard deviation, HPF: High-power fields

Table 5: Mast cell count across PNI

PNI	*n*	Mean	SD	Median mast cell count/10 HPF
Absent	32	39.97	7.835	39.00
Present	9	44.67	6.103	44.00
Total	41	41.00	7.675	40.00

P=0.08. SD: Standard deviation, HPF: High-power fields, PNI: Perineural invasion

Table 6: Serum histamine level across grades of tumor

Grade of tumor	*n*	Mean serum histamine (ng/ml)	SD	Median
Low	15	68.328	71.6658	42.700
High	14	52.414	61.0694	34.500
Total	29	60.646	66.0684	42.700

P=0.5. SD: Standard deviation

Table 7: Serum histamine level across PNI

PNI	*n*	Mean serum histamine (ng/ml)	SD	Median
Present	14	63.380	63.4091	60.200
Absent	15	58.093	70.5839	41.600
Total	29	60.646	66.0684	42.700

P=0.79. SD: Standard deviation, PNI: Perineural invasion

Table 8: Serum histamine level across lymphovascular invasion

LVI	*n*	Mean serum histamine (ng/ml)	SD	Median
Present	18	74.946	68.4003	60.200
Absent	11	37.245	57.4286	12.100
Total	29	60.646	66.0684	42.700

P=0.12. SD: Standard deviation, LVI: Lymphovascular invasion

Table 9: Serum histamine level across depth of invasion

Depth of invasion (mm)	*n*	Mean serum histamine (ng/ml)	SD	Median
<10	23	49.805	57.0525	12.100
≥10	6	102.200	86.6576	60.450
Total	29	60.646	66.0684	42.700

P=0.059. SD: Standard deviation

most important mediators involved in angiogenesis.[20] A study by Salem *et al*. noted a decrease in the staining intensity of histamine receptor protein (H4R) with increases in the histopathological grade of OSCC cases.[13] As the mast cell mediator histamine exerts its effects by binding to its receptors, it is suggested that with increased levels of histamine, the receptor levels go down and thus reflect an active role of mast cell and their mediators in higher grades of cancer. In the present study, serum histamine levels of the cases of squamous cell carcinoma increased with increase in the depth of invasion of the tumors [Table 9]. However, the levels of serum histamine did not correlate with the histopathological prognostic indicators such as grading, lymphovascular invasion and perineural invasion [Tables 6-8]. Similar results were obtained by Jaafari-Ashkavandi *et al*. who studied the mast cell mediator tryptase in oral cancer patients.[21] No statistically significant correlation was seen between serum mast cell tryptase levels and tumor stage, nodal involvement or metastases. A study by Yadav *et al*. showed statistically significant increases in mast cell tryptase and chymase levels in OSCC.[22] The exact role of the mast cell mediators in oral cancers still needs to be deciphered, as no strong association was obtained with the histological characteristics except the depth of tumor invasion.

Although the number of cases included in our study were limited, we did find statistically significant correlations of tumor mast cell counts with the histological grading, lymphovascular invasion, and depth of invasion of tumor. These results could be well substantiated with adequate follow-up data of the individual cases.

Conclusion

As per the results of our study, the mast cell count in cases of OSCC can be used as a prognostic indicator in conjunction with the other clinicopathological determinants of prognosis. This may help to utilize the mast cell–tumor interaction and immune-modulatory effects of mast cells to individualize treatment options for cancer cases. This can be possible with the help of large-scale studies conducted over a considerable study period, including follow-up data of the clinical course of oral cancer patients.

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Conflicts of interest

There are no conflicts of interest.

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