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Tumour Budding: A Promising Parameter in Oral Squamous Cell Carcinoma.

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ABSTRACT

Oral squamous cell carcinoma (OSCC), the fifth most common cancer worldwide. The major risk factor for this neoplasm is chronic exposure of oral mucosa to tobacco and alcohol. The presence of tumour budding at the invasive front (IF) has been reported as a promising prognosticator in OSCC. Tumour budding is defined as isolated single cancer cell or a cluster of cancer cells composed of fewer than five cells. The study aimed to identify the characteristics of tumour budding in prognostication of OSCC. Thirty histological specimens of biopsy proven OSCC were considered in this study. Serial sections of 4 um thickness were taken and stained. Cancer cells were observed in cancer-stroma lesions at the invasive front of the tumour. Number of tumour budding foci were counted in the histological fields in which the tumour budding intensity was maximal. Independent t test was used for comparing tumour budding with prognostic parameters on the basis value p value < 0.05 which was considered as statistically significant. The degree of tumour budding was linked with poor tumour differentiation. Presence of tumour budding foci is a significant indicator to predict the prognosis in Oral cancer patients.

Keywords: Tumour budding, Oral squamous cell carcinoma, Invasive front

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INTRODUCTION

Oral squamous cell carcinoma (OSCC), the fifth most common cancer worldwide, is a major cause of morbidity and mortality in India. The major risk factor for this neoplasm is chronic exposure of oral mucosa to tobacco and alcohol. The main clinical prognostic marker in OSCC is TNM staging. The presence of tumour budding at the invasive front (IF) has been reported as a promising prognosticator in OSCC.[1] Tumour budding is defined as isolated single cancer cell or a cluster of cancer cells composed of fewer than five cells.[2]

Those budding cells detached from the tumour bulk and migrated to the adjacent stroma. It represents a more aggressive and malignant potential of the tumour.Tumour budding is an expression of two properties of malignancy: loss of cellular cohesion and active invasive movement. It has been associated with poor prognosis in tongue carcinoma.[3] The study aimed to identify the indicators that would be used to predict prognosis of Oral Squamous Cell Carcinoma based on tumour budding.

MATERIALS AND METHODS

Thirty histological specimens of biopsy proven Oral Squamous Cell Carcinoma from were taken from Departmental archives after approval from Institutional ethics committee. Tissue sections of 4 um thickness were taken and stained with hematoxylin and eosin.

Tumour budding is defined as single cancer cells or clusters composed of up to four cancer cells. These cancer cells were observed in cancer-stroma lesions at the invasive front of the tumour. (Fig 1). Number of tumour budding foci were counted in ten high power histological fields and their average was taken. Number of cell in each tumour bud was counted. Tumour budding was compared with lymphnode and margin status, recurrence and survival of the individual. According to the number of tumour budding classified as low intensity group were budding intensity was < 5 and high intensity group were budding intensity was >5. Distance of the tumour bud was assessed using an eyepiece reticule from the bulk/body of the tumour. (Fig 2)

Statistical Analysis

Independent t test was used for comparing tumour budding with prognostic parameters on the basis of a value of $P < 0.05$ which was considered as statistically significant.

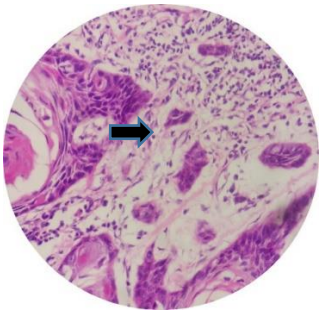

Fig 1: Tumour budding (➡) at the invasive front

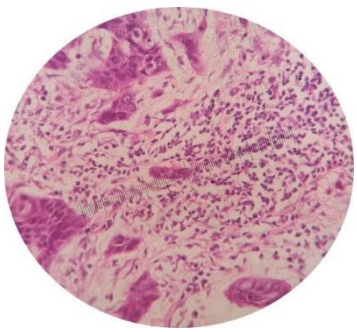

Fig 2: Measuring tumour depth using eye piece reticule

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RESULTS

The study population consisted of thirty cases (24 Males and 6 females) of histologically proven squamous cell carcinoma which included five cases of well differentiated 22cases of moderately differentiated and three cases of poorly differentiated squamous cell carcinoma with their average age between 45-80 years. On comparison of recurrence with tumour bud characteristics it was observed that recurrences cases had more number of tumour buds (30.88 µ +/- 10.13 µ) & tumour bud were also was further away with the farthest tumour bud at 3.92 µ +/-1.52 µ.The closest tumour bud was also further away 0.88 in recurrence cases & size of tumor bud was smaller when recurrence occurred 22.38 µ +/-5.041 µ. (Table 1).

On based on death or survival of patient total number of tumour buds was higher (31.8µ+/- 11-64 µ) in those who were dead while compared to the ones who survived. The total number of cells in the smallest tumour bud was lesser (21.2 µ +/- 6.26 µ) in the ones who were not alive. On comparison with the distances of farthest tumour bud in patients who were dead was more (4.31 µ +/- 1.61+/- µ) when compared to those who were alive. The distance of closest tumour bud was 0.96 µ +/- 1.14 in the cases who did not survive. (Table 2).

On comparison on the status of margin which was free or involved the total number of tumour bud in involved cases were 26.6 µ +/- 8.98 µ. The total number of cells in the smallest bud was 22.8 µ +/- 7.95 were margins were involved. The distance of the farthest tumour bud was 3.42 µ +/ - 1.07 µ in cases were margins were involved. The minimum distance of tumour bud were margins were involved was 0.48 µ +/- 0.06 µ. (Table 3)

Table 1: Comparison of recurrence with tumour bud character

	Recurrence	N	Mean	Std. Deviation	t	df	P VALUE
Total number of tumour bud	NO RECURRENCE	10	25.4	9.276	-1.195	16	0.25
	RECURRED	8	30.88	10.134			
Total number of cells in the smallest bud	NO RECURRENCE	10	25.3	6.848	1.007	16	0.329
	RECURRED	8	22.38	5.041			
Distance of the farthest tumour bud	NO RECURRENCE	10	3.463667	1.177726	-0.72	16	0.482
	RECURRED	8	3.92125	1.524593			
Minimum distance	NO RECURRENCE	10	0.82	0.5287	-0.194	16	0.849
	RECURRED	8	0.888	0.9357			

Table 2: Comparison based on death or survival of patients along with tumour bud character

	survival	N	Mean	Std. Deviation	t	df	P VALUE
Total number of tumour bud	ALIVE	13	26.31	9.013	-1.072	16	0.3
	DEAD	5	31.8	11.649			
Total number of cells in the smallest bud	ALIVE	13	25.08	5.965	1.22	16	0.24
	DEAD	5	21.2	6.261			
Distance of the farthest tumour bud	ALIVE	13	3.416667	1.167785	-1.325	16	0.204
	DEAD	5	4.318	1.611388			
Minimum Distance	ALIVE	13	0.808	0.5283	-0.395	16	0.698
	DEAD	5	0.96	1.1437			

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Table 3: Comparison based on status of margin involved along with tumour bud character

	ln status	N	Mean	Std. Deviation	t	df	P VALUE
Total number of tumour bud	FREE	7	26.29	9.604	-0.069	15	0.946
	INVOLVED	10	26.6	8.984			
Total number of cells in the smallest bud	FREE	7	23.71	4.461	0.274	15	0.788
	INVOLVED	10	22.8	7.955			
Distance of the farthest tumour bud	FREE	7	3.205714	1.632992	-0.342	15	0.737
	INVOLVED	10	3.429	1.074062			
Minimum distance	FREE	7	1.071	1.0177	1.535	6.032	0.175
	INVOLVED	10	0.48	0.0632			

DISCUSSION

Tumour budding has been considered as an important histopathological parameter for evaluating the malignant degree and prognosis of oral cancer. Invasion is one of the hall marks of oral cancer which determines the progression and metastases.A modification in the pattern of invasion was introduced by Chang et al(2010) which is known as tumour budding. It is defined as the presence of single cells or small cluster of cells (<5 cells) at the invasive front. Tumour budding represents cells that have lost their cohesive properties[1]. In the present study we found that tumour budding was considered to be an adverse prognostic parameter. According to Nan Xie etal [4] high intensity tumour budding indicates poor prognosis of patients such as cervical lymphnode metastases, reduced survival. High intensity of tumour budding and deeper invasive depth correlated with reduced overall survival and tumour budding is a parameter independently predicting the prognosis of patients with oral cancer.[4]

Tumour budding is considered as a marker of many important events in in oral carcinoma which include: epithelial-mesenchymal transition, invasion, metastasis, and subsequent prognosis.[5] In this study we analyzed the correlation of depth of invasion and tumour budding characteristics with other parameters of prognosis of patients .We found that high intensity of tumour budding and deeper invasive depth correlated with reduced overall survival. Many studies showed that cervical lymphnode metastases increased when depth of invasion exceeded 4mm.[4]

Tumour budding is defined as undifferentiated signal cancer cell or small cluster composed less than five cancer cells which was introduced by Vierra M. These buds represents a more aggressive and malignant phenotype of tumour cells. Tumour budding may represent cells undergoing epithelial – mesenchymal transition (EMT). Chang et al described the EMT as the process by which cells undergo a switch from a polarized, epithelial phenotype to a motile mesenchymal phenotype.[6]

It was suggested by Wang et al based on low expression of the cell adhesion molecule E-cadherin at the budding site. Loss of E – cadherin expression is involved in epithelial – mesenchymal transition (EMT) and E – cadherin is therefore emerging as one of the caretakers of the epithelial phenotype.[7] E- cadherin is required for the maintenance of stable junctions:anti E-cadherin in antibodies can disrupt these contact and induce a mesenchymal phenotype which is associated with invasive behavior. The expression of E- cadherin varied within the tumour cell population with tumour cells at invasive tumour front showing pronounced reduction in expression, while those closer to the surface retained their epithelial characteristics. Studies have proved that solitary cells originating from budding sites are positive for β catenin which can lead to EMT, resulting in these cells becoming solitary invasive cells.[7]

In oral squamous cell carcinoma a strong correlation between high activity of tumour budding and increased density of stromal alpha smooth muscle actin positive myofibroblasts have been reported to influence tumour proliferation and metastases[1].According to F. Mahomed et al[8] the reduced expression of E- Cadherin in OSCC was less at tumour proper and was further reduced at the invasive front.[8]

The increased tumour bud distance and reduced tumour bud size were significantly associated with increased metastases. The farther the tumour buds, the greater the level of EMT that was taking place. Thus, it indicates that EMT plays a significant role in the process of metastasis, which is directly associated with the post-translational changes following down regulation of E- Cadherin. Loss of E-Cadherin directly reflects the loss of the adherens junction leading to loss of cohesion.[9] E-Cadherin also promotes matrix metalloproteinases which along with TGF-β stimulated MMP9 can degrade basement membrane leading to initiation and further invasion of tumour.

Tumour budding is easy to identify and is reproducible. In routine diagnosis we can identify the number of tumour budding cells and categorize the patients into high- risk group and low- risk group and provide optimal treatment . Thus, elective lymphnode dissection and adjuvant radio therapy may be beneficial for patients with high intensity tumour budding regardless of their TNM stage. Therefore tumour budding may be used as a reliable parameter and applied to make decision of optimal therapy.

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