Original Article

Pattern of invasion as a factor in determining lymph node metastasis in oral squamous cell carcinoma

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ABSTRACT

Context: Lymph node metastasis (LNM) influences survival of oral squamous cell carcinoma (OSCC). Evidence supports the value of prognostic information provided by most aggressive cells that lie in the tumor invasive front.

Aims: This study evaluated the clinical and histological parameters (C and HP) that would best associate with LNM in OSCC.

Settings and Design: A review of records and histological examination of nonrecurrent surgically treated 182 cases.

Subjects and Methods: A review of records and histological examination according to the Bryne's invasive front grading system of nonrecurrent surgically treated 182 cases (pN– =100; pN+ =82) was undertaken. The data were subjected to suitable statistical analysis to check the agreement between observers, association of the parameters to LNM, and to identify the best among all of them.

Statistical Analysis Used: Kappa statistics, Pearson's Chi-square, Fisher's exact test, multiple logistic regression analysis.

Results: None of the C and HP, with the exception of pattern of invasion (PI) ($P = 0.000$), modified degree of keratinization and nuclear polymorphism ($P = 0.041$, 0.022), and total malignancy score for survival ($P = 0.013$) showed a significant association with nodal status. PI was identified as the most influencing parameter of all.

Conclusions: Factor that is primarily the manifestation of tumor and its microenvironment has taken the prime seat followed by the ones that are dictated by the tumor. The factors that are basically quantified were not able to show association. Site influences the nodal status alongside PI.

KEY WORDS: Bryne's invasive front grading system, clinical parameters, histopathological/morphological parameters, lymph node metastasis, oral squamous cell carcinoma

INTRODUCTION

Numerous prognosticators have been identified for oral squamous cell carcinoma (OSCC). Reported survival of patients with lymph node metastasis (LNM) after surgery is 20–30% compared to 63–86% in LNM-free patients.[1] Staging neck lesions by palpation often yield false-negative result while computed tomography/magnetic resonance imaging better enable the detection.[2] In clinically, node negative cases with treatment, 75–77% cases remain tumor-free on histopathological examination[3,4] while in those without treatment, 32% develop LNM.[5,6] indicating the value of nodal status as an important prognosticator.[7]

This study sought to use clinical and histological parameters (C and HP) of the primary OSCC lesion to assess the association with regional LNM and to identify the most influencing parameter.

SUBJECTS AND METHODS

A total of 182 cases (pN– =100; pN+ =82) of surgically treated, nonrecurrent OSCC having no previous history of any malignancy have been included in the study. Our study considered using clinical parameters such as age, gender, site, size, clinical growth pattern (CGP), premalignant status (PMS), habits, and histological parameters such as Bryne's invasive tumor front scoring system (invasive front grading [IFG]) and its

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individual histomorphological variables in determining regional LNM.[8] The histopathological parameters were analyzed by two pathologists independently. Some of the representative morphological variations have been shown in Figure 1. Lesions arising from or showing extensions into oropharynx/nasopharynx, lip-skin surface, and lesions that are of intra-osseous origin were not considered. Tumors of all sizes were included, wherein a minimum of at least ten nodes was examined histologically.

The reproducibility of the scoring system by pathologists was calculated by Kappa statistics. Categorical data forming the frequency tables were analyzed for their association with nodal status by Pearson's Chi-square or Fisher's exact test. Multiple logistic regression analysis was performed to know the most influencing parameter on nodal status among all. The best regression model that would show the percentage of predictability of the nodal status was derived.

The study was performed in accordance with the Declaration of Helsinki, and informed consent was obtained from patients who were included in the study.

RESULTS

Oral squamous cell carcinoma observations

In our study, about 30% of the individuals were <40 years of age (ranging 27–39 years), while rest were ≥40 years of age (ranging 40–72 years). The mean age of patients with gingival/alveolar process carcinoma was higher than those from other sites (56 years against 48–50 years). The mean age of females affected by OSCC was slightly higher than males (52 years against 48 years). Males are more commonly involved and even in the most common site-buccal and alveolar mucosa ($n = 129$), the ratio was as high as 8:1. The size of the lesion most often recorded ranged from >2 to <4 cm (~60%), followed by ≥4 cm (~30%) and only a few were ≤2 cm (10%) [Table 1].

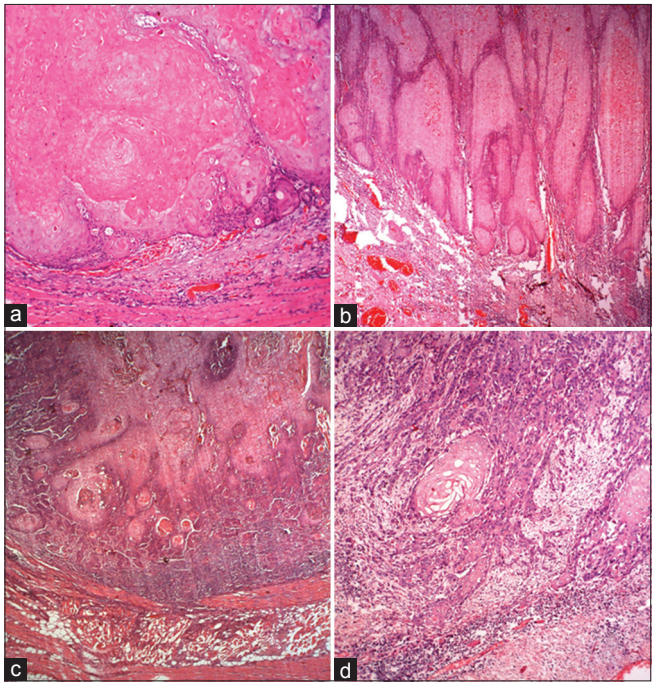

Figure 1: (a-d) Representative micrographs from squamous cell carcinoma tissues displaying four patterns of invasion as described in the invasive front grading system (pattern of invasion = 1 to pattern of invasion = 4 from 1a to 1d, respectively). (H and E, ×4)

Statistical observations

The histopathological parameters in our study, when subjected to Kappa statistics, showed a significant inter- and intra-observer agreement ($P = 0.00$). The intraobserver values remained higher/identical for each parameter compared to the interobserver values except for nuclear polymorphism (NP). A "good" (0.61–0.80) to "very good agreement" (>0.80) was obtained for each of the parameters analyzed. The interobserver agreement was the highest for pattern of invasion (PI) and at intraobserver level, it was for the degree of keratinization (DK) [Table 2].

None of the clinical parameters [Table 1] and histological parameters except PI ($P = 0.00$) and total malignant score for survival (TMS(S)) ($P = 0.01$) [Table 3] showed association to pN. Modification of scores[9] of individual parameters yielded association for DK (DK-mod) ($P = 0.04$), NP (NP-mod) ($P = 0.02$), and PI (PI-mod) ($P = 0.00$), but not for the number of mitosis (NM-mod) and lymphoplasmacytic infiltration (LI-mod). The regression equation containing five variables – NP-mod, TMS (S), LI-mod, site, and PI – gave best classification table with a percentage of predictability of 65.9% [Table 4]. PI emerged as the single most influencing parameter on the nodal status but with lower predictability (62.1%). The site was the last parameter to be removed and equation containing these two variables (PI and site) gave a predictability of 63.2%.

DISCUSSION

The study tried to find the association of various C and HP to the nodal status and to see the association of prognostic groups of tumor at the invasive front to the same. This becomes important because many tumors, in this part of the world, occur in buccal mucosa and patients report to the health-care centers only after a considerable growth has been attained, unlike small tumors recorded in the tongue and floor of the mouth among the Western population on which abundant information on the prognosticators is available.[3,10-12]

Literature review does not yield a consensus in regards to clinical course or to the prognosis of OSCC in younger versus older patients'.[13] Few studies have stated that the survival or control of the disease is poor in younger individuals.[14-16] Thus, although age was selected as one of the parameters, considering younger individuals may show an aggressive progression of disease, in this case, the nodal involvement, no association with nodal status was elicited.

Table 1: Distributional frequency of clinical parameters analyzed with their statistical association to nodal status

Clinical parameters	Categorical description	Nodal status		Total	*P* value
		pN−	pN+		
Age	<40	27	21	48	0.87
	≥40	73	61	134	
Gender	Male	87	63	150	0.33
	Female	15	17	32	
Site	Lip-mucous membrane	1	3	4	0.77
	Tongue	15	10	25	
	Cheek (buccal mucosa and alveolar mucosa)	71	58	129	
	Floor of mouth	3	2	5	
	Alveolar process (gingiva)	10	9	19	
Size	≤2 cm	10	9	19	0.87
	>2 cm but <4 cm	62	52	114	
	≥4 cm	28	21	49	
Clinical growth pattern	Exophytic	46	41	87	0.66
	Endophytic	54	41	95	
Premalignant status	Not documented in the data	65	46	111	0.53
	Leukoplakia	6	4	10	
	Oral submucous fibrosis	11	11	22	
	Erythroleukoplakia	7	5	12	
	Erythroplakia	11	16	27	
Habits	No habits	14	15	29	0.87
	Chewing without tobacco <5 years	1	2	3	
	Chewing without tobacco ≥5 years	12	13	25	
	Chewing with tobacco <5 years	10	6	16	
	Chewing with tobacco ≥5 years	39	30	69	
	Combination <5 years	2	1	3	
	Combination ≥5 years	22	15	37	

Table 2: Kappa values for the various histological parameters analyzed

Histopathological parameters	Kappa values	
	Intra-observer	Inter-observer
Degree of keratinization	0.88	0.79
Nuclear polymorphism	0.77	0.81
Number of mitoses	0.86	0.77
Pattern of invasion	0.84	0.84
Lympho-plasmacytic infiltration	0.88	0.80
Total malignancy score	0.69	0.59

Rate of nodal involvement, although reported for oral cavity, varies from 35.3% to 60% among OSCC patients, 37–57% of the tongue, 11–50% of the mandibular gingiva, and 24–75% of the buccal mucosa show nodal metastasis[17] indicating the importance of recording this as a parameter. In addition, studies show[10] tumors of the tongue, retromolar area, and oropharynx often is histologically diagnosed nodal positive (59–64%), but buccal and gingival tumors/alveolar tumors do not (only 22% and <7%, respectively). Although independently site did not show association with pN, this was the last parameter to be removed allowing PI to emerge into an influencing parameter in logistic regression imparting an addition dimension to PI itself in a given site.

The value size as a parameter is quite evident in the proposed tumor, node, metastasis (TNM) staging with stage ascending regularly in spite of the absence of extension of disease into other regions of the body (both regional and distant sites). Studies have shown that larger size of the primary tumor is associated with poorer prognosis and poor survival rate.[1]

Unlike the exophytic tumors that grow out from the mucosal surface and rapidly become symptomatic, the endophytic tumors are usually hidden from view and may be quite extensive at the time of diagnosis. The diagnosis of endophytic tumors is thus more difficult and is usually delayed, affecting the prognosis of the tumor. It has been suggested that endophytic tumors have a higher propensity to metastasize to cervical lymph nodes than exophytic tumors.[18] Thus, CGP in our study was recorded as exophytic and endophytic.

Lesions in young patients are predominantly invasive compared to the exophytic lesions found in older patients. This often suggests that the biological behavior of OSCC in the young may be distinct from that occurring in older people. The higher propensity for endophytic growth in the younger population may reflect the higher incidence of LNM and less favorable response to treatment in them.[19] In our study, while both CGP was equally seen in ≥40 years of age subset of patients, there was a slight increase (58%) in the number of endophytic CGP in <40 years patients. Further, the proportion of endophytic tumor that was node positive (12/48 or 25%) was higher than the proportion of exophytic tumors (9/48 or 19%), showing the same in younger individuals. In ≥40 years of age patients, the proportion of endophytic tumors (22/134 or 22%) who were node positive was only slightly lesser than the proportion of exophytic tumors (32/134 or 24%) exhibiting the same.

In the most common site involved – buccal mucosa – the number of cases that displayed a size <3 cm was fewer and the same condition prevailed in the floor of the mouth where

Table 3: Distributional frequency of histological parameters analyzed with their statistical association to nodal status

Histological parameters	Categorical description	Nodal status		Total	*P* value
		pN−	pN+		
Degree of keratinization (DK)	1	11	4	15	0.16
	2	30	17	47	
	3	27	27	54	
	4	32	34	66	
Nuclear polymorphism (NP)	1	6	4	10	0.10
	2	31	13	44	
	3	32	30	62	
	4	31	35	66	
Number of mitoses (NM)	1	63	49	112	0.91
	2	17	17	34	
	3	10	7	17	
	4	10	9	19	
Pattern of invasion (PI)	1	13	3	16	0.00
	2	26	5	31	
	3	18	18	36	
	4	43	56	99	
Lympho-plasmacytic infiltration (LI)	1	12	4	16	0.09
	2	45	33	78	
	3	40	37	77	
	4	3	8	11	
Total malignancy score for survival [TMS (S)]	5 to 8	14	3	17	0.01
	9 to 12	33	19	52	
	13 to 20	53	60	113	
	9 to 12	38	27	65	
	13 to 16	39	48	87	

Table 4: Displays variables in the equation and the best logistic classification table obtained

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 6						
Constant	1.221	1.131	1.165	1	0.28	3.39
Site			3.915	3	0.271	
Site (1)	1.528	1.674	0.833	1	0.361	4.61
Site (2)	−1.083	1.172	0.855	1	0.355	0.338
Site (3)	−0.729	1.105	0.434	1	0.51	0.483
PI			12.288	3	0.006	
PI (1)	−3.048	1.224	6.201	1	0.013	0.047
PI (2)	−2.69	0.811	11	1	0.001	0.068
PI (3)	−0.526	0.528	0.994	1	0.319	0.591
NP_MOD (1)	−0.247	0.564	0.192	1	0.662	0.781
LI_MOD (1)	−0.447	0.333	1.797	1	0.18	0.64
TMS_S			1.569	2	0.456	
TMS_S (1)	1.517	1.267	1.435	1	0.231	4.56
TMS_S (2)	0.679	0.672	1.022	1	0.312	1.972

The cut-off value is 0.500

Nodal status	Predicted negativity	Predicted positivity	Total
True negativity	58	42	100
True positivity	20	62	82

all of the five recorded cases showed ≥3 cm size. It is to say that many cases recorded in the study were of larger size. The subsets of tongue tumor measuring <3 cm in node negative and positive cases were considerably different (4/6 or 67% and 2/6 or 33%, respectively), but this difference was not so in the tumors measuring ≥3 cm (11/19 or 58% and 8/19 or 42%, respectively). Interestingly, this difference was not much farther apart in the tumors of buccal mucosa and alveolar process/gingiva for both subsets of sizes (5/12 or 42% and 7/12 or 58% for <3 cm; 76/136 or 56% and 60/136 or 44% for ≥3 cm). This highlights the impact of site over the size of the tumor on nodal status assessment even if they have not shown association individually. A similar situation was earlier explained for age and CGP. Furthermore, we feel many parameters may influence the tumor indirectly in a complex manner, especially in large tumors as shown by us.

Another likely possibility for the lack of correlation between the size of the primary tumor and nodal status may be due to the fact that size is just the measurement of the surface greatest dimension and is restricted to indicate tumor size in the TNM staging classification system. Factors such as, "tumor thickness" are now recognized to be a more accurate histological prognosticator.[20]

The heterogeneous cell population that constitute SCC shows differences in invasive and metastatic behavior; thus, there was a reported lack of correlation that existed between Broders' grades and the prognosis of head and neck SCC.[11] The cells at the most advanced parts of a carcinoma, namely, tumor's invasive front, possess characteristics different from the central cells. Histologically, these parts contain cells that have the ability to invade the surrounding tissues and structures, including vessels, and thereby to metastasize.[21] These cells are also more poorly differentiated indicating increased aggressiveness of tumor at this site compared to the whole tumor.[12,22] Thus, cells of the whole tumor may offer no prognostic value, in contrast to, the cells at the invasive front. Bryne's IFG system has been shown to be of high, independent prognostic value for OSCC.[12]

Since each morphological parameter in the system correlates with prognosis and agreement of each of these was not found to be better than the system as a complete unit in itself, omitting a factor may not offer a better result.[8] Our study has made an attempt to improve the agreement between the observers by several methods without eliminating any specific histological parameter. The measures taken to improve the agreement were precalibration between the observers, preanalyzing 50 cases before the current evaluation, and by evaluating NP under higher magnification as suggested for NM by Bryne himself.[8]

The differentiation of the tumor cell population is expressed by the DK, NP, NM, and ability of the tumor cells to maintain cohesiveness so as to keep the tumor cell population together.[11] Keratin, an intermediate filament-forming protein, contributes to the maintenance of cell shape through its three-dimensional organized structure. This framework organizes during maturation of epithelial cells.[23,24] Low-grade tumors were seen to be well differentiated with increased keratinization and thus, histologically appear most similar to the parent tissue from which they arose. The high-grade tumors were poorly differentiated and highly anaplastic and showed no evidence of keratinization.[11,25] Several studies have provided evidence supporting an active keratin role in cancer cell invasion and

metastasis.[23] This may be by increased deformability through influence on the cell shape, higher migratory ability of epithelial cells through interactions with the extracellular environment, or through chaperone-mediated intracellular signaling.[26-28]

Cell differentiation during embryogenesis undergoes extensive structural reorganization within nucleus, making naive stem cells more pliable than differentiated cells[29] and thus more differentiated cells in tumor exhibit no or less NP.[11] Also we know, in cyclic divisions that take place within a cell, mitosis is the only histologically distinguishable phase.

While DK showed correlation to LNM in tongue[30,31] and pharyngeal and laryngeal carcinomas,[32] NM did not do so. In our study, DK, NP, and NM did not show correlation to LNM. On scoring modification, DK and NP showed a significant correlation to LNM. This was supported in our data observation itself where tumors that display higher DK (66%) and lower degree of NP (69%) were seen in node negative cases compared node positive cases. However, this difference was not so marked in cases with lower DK and higher NP [Table 3].

Interactions between cells and their microenvironment are mediated by adhesion molecules that participate in fundamental biological processes, including cell migration among others. Thus, metastasis is mediated by the alterations within tumor cells in coordination with nonneoplastic stromal cells.[33] The role of underlying stromal microenvironment in growth, differentiation, and metastasis of tumor epithelial cells comes from the studies undertaken in urogenital tissues of rat model and such a influence in carcinoma cells by stroma could just be the extension of postnatal mesenchymal inducer role.[34] While differentiated neoplastic cells have the tendency to invade the underlying connective tissue with pushing, well-delineated borders, the poorly differentiated cells of the tumor possess significantly infiltrative margins.[35] A significant correlation was found between the frequency of metastases and the type of invasive growth pattern.[11,35] The importance of the same is evident from verrucous carcinoma, nodular/pigmented, and superficial variants of basal cell carcinoma versus the poorly differentiated SCC and morpheaform/infiltrating forms of basal cell carcinoma. Clearly, this was evident in our study finding through the significant association that PI showed with LNM. This kind of association was demonstrated earlier in tongue[30,31] and not in oropharyngeal carcinomas.[32]

Much debate exits on whether the inflammatory sequel to tumor development is a host immune response or is a result of the influence of the tumor itself on the host stromal tissue. Thus, the role of the inflammatory cells in tumorigenesis remains unclear; whether they promote or demote the progression of cancer. It is possible that a self-amplifying loop is established between the tumor cells and the inflamed stroma that was initially set by tumor which later goes to reciprocate by enhancing the malignant traits in the tumor cells.[33] LI did not show association in our study to LNM similar to the findings by others.[30-32]

Logistic Regression analysis was performed to arrive at the most influencing parameter of all. The analysis was begun with all the parameters except age, gender, PMS, and habits since we felt that these may contribute in the initial progression of the disease rather than the later stages. Inspite of the presence of very many variables, the percentage of predictability was only 63.2–64.3% until step 5. The best regression model including TMS (S) as a component along with PI, NP (mod), LI (mod), and site offered a better predictability (65.9%) which further goes to considerably drop when only PI emerged as the most influencing factor (62.1%). A good level of agreement was achieved in our hands both at intra- and inter-observer levels for PI and with site as an added detail increases the predictability by 63.2%. Although the level of predictability of these parameters was less, refining the form, and depth of penetration of the tumor in a given site will help us in future to achieve this.

CONCLUSIONS

PI emerging as the most influencing parameter reinforce that tumor and its progression is a definitive manifestation of a finely balanced dynamic interplay between the tumor and host microenvironment. The factors such as DK and NP influence less on nodal status and require modification compared to PI since they only define structural and the functional characters of the tumor cells. This is further supported by their elimination at early stage from regression sequence. NM and LI according to us represent a complexly controlled process while the former is only visualized partly by routine histopathology, the latter factor is a constantly evolving/modifying micro-environment; thus, both of them cannot be simply defined by the quantification for proving their association. Although no clinical parameters showed an independent association, glimpses of their complex association with nodal status have been felt and have been presented in the study. It is important to state that site was the last parameter to be eliminated from the sequence indicating the potential influence of it alongside PI on the nodal status among all the others. Thus, factors that describe the site specifically and more detailed diversification of PI could contribute to increased predictability.

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Conflicts of interest
There are no conflicts of interest.

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