Perineural Invasion in Oral Squamous Cell Carcinoma in Relation to Tumor Depth

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Background: The American Joint committee on Cancer in their 8th edition staging manual regarded perineural invasion as one of the most important prognostic factors for Lip and Oral Cavity Squamous Cell Carcinoma, it also incorporated tumor depth of invasion in defining tumor size category in the new staging system. This study was conducted to evaluate the frequency of perineural invasion in oral squamous cell carcinoma and the effect of approaching tumor depth in this process.

Materials and Methods: fifty-four formalin fixed paraffin embedded tissue blocks of radical resections of Oral Squamous Cell Carcinoma were cut and stained with Hematoxylin and Eosin stain, then evaluated for perineural invasion, with estimation of tumor depth of invasion for each case.

Results: Perineural invasion was found in twenty-two cases of the study sample. The diameter of the largest nerve bundle that showed perineural invasion was found to have a positive significant correlation with tumor depth of invasion (p=0.025). Perineural invasion status in terms of (present, absent) showed a significant difference with patients' age (p=0.037), also showed a significant association with tumor site (p=0.004), however, this association was non-significant in regard to tumor grade and stage (p=0.848, p=0.520) respectively.

Conclusion: The attacking potential of preceding tumor depth and those cancers affecting young individuals may be reflected by the presence of neural infiltration by tumor cells. Tongue resected tumors should be carefully inspected for this deceptive biological process.

Keyword: Carcinoma, tumor. (Received: 15/9/2018; Accepted: 15/10/2018)

INTRODUCTION

Perineural invasion (PNI) can be broadly defined as the dissemination of carcinoma along or within a nerve^[1]. It is characterized by tumor cells presence in the perineural space^[2]. Its incidence is about two thirds of the cases of head and neck cancers, with one third being associated with Oral Squamous Cell Carcinoma (OSCC)^[3]. PNI is considered as one of the four routes of tumor metastasis in several human neoplasms, which is associated with poor prognosis especially those in the head and neck area^[4].

The worse outcome of a tumor was found to be correlated with the invasive potential of tumor cells. In one of the researches, it was postulated that the offensive tumor course can be manifested by its downward growth below an imaginary line from the adjacent healthy oral mucosal basement membrane, in which below such line the tumor have to destroy the tissues beneath in order to establish invasion. For an exophytic type of tumors, this outward growth may be mistaken in assessing tumor depth as it doesn't truly demonstrate the ability of the tumor to overcome the resistance of the underlying tissues. On the other hand, when having ulcerated endophytic tumors, the lost part due to ulceration should be considered as it represent the tissue that have been destroyed by the tumor in order to

invade deeper structures^[5]. Therefore, tumor depth of invasion (DOI) can be defined as the extent of tumor growth into the tissues deeper below the surface epithelium^[6].

Its more reliable to assess the depth from the basement epithelium as tumor spread into a regional lymph node actually occur only when the tumor crosses the basement membrane^[7]. That's why tumor DOI is deemed to be a more acceptable histological feature as occult nodal metastasis can be best predicted by this invasive $action^{[8,9]}$. It is well known that the invasive front of a tumor, by which the most aggressive cells reside; can reflect the prognostic information related to that tumor^[10]. The admission of tumor DOI was done recently for defining tumor size in the American Joint Committee on Cancer (AJCC) 8th edition of staging system for OSCC, considering that for each increase of 5 mm in DOI, both clinical and pathological T categories will be stepped one level interval^[11].

This study aimed at histopathological evaluation of PNI in OSCC with evaluation of advancing tumor DOI on this biological process.

MATERIALS AND METHODS

Fifty-four formalin fixed paraffin embedded tissue blocks of radical resections of OSCC were retrieved from the archives of the department of

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each case was cut and mounted on normal glass slides, stained with hematoxylin and eosin (H&E) for histopathological evaluation. Tumor grading based on Broders classification and staging in accordance with the recent uploaded 8th edition of staging system for Lip and Oral Cavity Cancer were used^[12,13]. Other clinicopathological data including patients age and site of the tumor that were present in the relevant surgical reports were also included. In this study, imageJ program was used for measuring histopathological tumor DOI, in addition to the diameters of neural foci that showed PNI.

Microscopical tumor DOI was obtained by measuring "the length of a plumb line that was drown form the level of the basement membrane of the adjacent normal oral mucosa to the deepest invasive point of the tumor"^[13]

According to Liebig's definition, PNI was identified "when the tumor cells were seen within any of the three layers of the nerve sheath and/or tumor cells were seen in close proximity to the nerve and encircling one third or more of its circumference"^[4]. For every case that was positive for PNI, the diameter of the largest nerve bundle was measured at high magnification (40X) with total number of PNI foci estimation. Further PNI criteria including the density of the foci weather being single or multiple in addition to the site and pattern of the largest nerve showing PNI foci were also assessed. The site was classified into intratumoral (for those foci located inside the tumor), peripheral (for those foci located about 0-0.2 mm from tumor edge), and extratumoral (for those foci located beyond tumor edge)^[14,15].

Statistical analyses were achieved by using SPSS statistical package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA). All quantitative data were tested using Student t-test and ANOVA for differences between groups. Pearson's correlation for relation between variables; while qualitative relations were tested using Chi-square test. P value of <0.05 was considered statistically significant.

RESULTS

Patients mean age was (56.43 ± 14.27) ranging from (22-85) years. The tongue was the most common site constituting nearly half of the cases Dentistry/Baghdad University. 4 µm thickness for

(25 cases), followed by the remaining sites. Average tumor DOI in millimeters was 6.4 ± 3.2 (ranging from 1.1-14.5 mm).Regarding the grade, more than half (32 cases) were of well SCC, followed by the other patterns. Stage IV was the most frequent (29 cases), followed by the other stage categories (Table 1).

About half of the cases (22 cases) of the study sample were positive for PNI, the diameter of the largest nerve that showed PNI was ranging between (0.026-0.379 mm) with a mean of 0.135 mm. into which 15 of them were of intratumoral location, and most of them showed a partial encirclement pattern of tumor cells (19 cases), (Fig. 1.A & 1.B). The overall number of PNI foci was ranging from 1-10 for each case, where the multiple foci (15 cases) were about twice as the single PNI foci (7 cases), (Fig.2). A significant positive correlation between the largest nerve diameter that showed PNI and tumor DOI was found (r=0.477, p=0.025), (Fig. 3), however, no significant difference was found between the largest nerve diameter showed PNI and tumor grade, stage (p=0.276, p=0.398) respectively, (Table 2).

With respect to PNI status (present, absent), there was a significant difference between PNI status and patients' age (p=0.037), which can be manifested as younger patients have PNI in comparison to older individuals, while this status showed no significant difference in regard to tumor DOI (p=0.181). There was a significant association of PNI status and tumor site (mainly the tongue) (p=0.004), however, this association was nonsignificant in regard to tumor grade and stage (p=0.848, p=0.520) respectively, (Table 1)

Tumor DOI was subcategorized into 3 groups based on the newly released staging system for the Lip and Oral Cavity SCC tumor DOI cutoff point into which group A: tumor depth \leq 5mm, group B: tumor depth \geq 5 \leq 10mm, and group C: tumor depth \geq 10mm. In this study group A represent 19 cases (35.18%), group B was 27 cases (50%), and group C was 8 cases (14.81%). It was found that the DOI subgroups was significantly related to the largest nerve diameter that showed PNI (p=0.036), (Table 2).

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Figure 1: Histopathological PNI in: (A) Intratumoral nerve showing complete encirclement pattern of tumor cells (arrows).
(B): Extratumoral nerve showing partial encirclement of tumor cells (arrow); (x400); N: nerve bundle; Scale Bar 100 µm.



Figure 2: The distribution of the site, pattern of the largest nerve diameter showing PNI and type of PNI foci of the study sample.



Figure 3: Pearson correlation between H&E largest nerve diameter showed PNI and tumor depth of invasion.

Table 1: Perineural invasion status (present, absent) in relation to patients age, tumor site, DOI, grade and sta	age.
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		Ν	PNI		Test	p value
			Present	Absent		
			Count(%)	Count(%)		
Age	Mean±SD		51±16	60±12	Student t- test	0.037*
	Minimum		22	38		
	maximum		79	85		
Site	Tongue	25	17(77.3)	8(25)	Chi square	0.004*
	Floor of the mouth	5	2(9.1)	3(9.4)		
	Buccal mucosa	7	2(9.1)	5(15.6)		
	Mandible	7	1(4.5)	6(18.8)		
	Maxilla	6	0	6(18.8)		
	Lower lip	4	0	4(12.5)		
DOI	mean±SD		7.122±3.118	5.928±3.224	Student t- test	0.181
	SE		.664	.570		
Grade	Well	32	14(63.6)	18(56.3)	Chi square	0.848
	Moderate	17	6(27.3)	11(34.4)		
	Poor	5	2(9.1)	3(9.4)		
stage	Ι	3	2(9.1)	1(3.1)	Chi square	0.520
	II	11	4(18.2)	7(21.9)		
	III	11	6(27.3)	5(15.6)		
	IV	29	10(45.5)	19(59.4)		

* Significant relation; P>0.05 not significant; DOI, depth of invasion; PNI, perineural invasion; SD, standard deviation; SE, standard error.

		Ν	Largest nerve diameter showed PNI	Test	P value
			mean±SD		
Grade	Well	14	.145±.103	ANOVA	0.276
	Moderate	6	.089±.045		
	Poor	2	.202±.112		
Stage	Ι	2	.113±.058	ANOVA	0.398
	II	4	.130±.062		
	III	6	.087±.047		
	IV	10	.170±.120		
DOI subgroups	Group A	6	.100±.057	ANOVA	0.036*
	Group B	12	.117±.070		
	Group C	4	.239±.140		

 Table 2: Largest nerve diameter that showed PNI in mm in relation to tumor grade, stage, and DOI subgroups.

* Significant relation; P>0.05 not significant; DOI, depth of invasion, PNI, perineural invasion; SD, standard deviation.

DISCUSSION

The evaluation of H&E slides of the study sample revealed the presence of 22 cases out of 54 that were positive for PNI. In fact only five of them were previously mentioned to have neural invasion in their relevant histopathological reports, proving that upon careful examination of histological slides of OSCC, PNI detection frequency will be surprisingly raised if it was taken seriously into consideration. The presence of hitopathological PNI mimics, together with subjective interobserver variations and different imprecise definitions of PNI in literature all were contributing factors affecting the differences in incidence of PNI detection^[16,17].

Miller et al. (2012) found that whenever PNI is located beyond tumor edge, a decreased recurrence time would be expected^[18], yet the recent AJCC staging system in their 8th edition for Lip and Oral cavity SCC considered both PNI foci (intra and extratumoral) as prognostic factors, moreover recommended to report the diameter of the largest nerve bundle that is involved by PNI for those multifocal extratumoral foci in pathology reports^[13]. The pattern by which tumor cells reside in the neuronal sheath may vary accordingly with respect to multiple histological sections and it was found of no prognostic value^[19,20].

Tumor recurrence, nodal spread as well as survival rates of patients affected by SCC were found to be influenced by the caliber of nerves that show PNI^[21]. In a study of cutaneous SCC, it was found that if the affected nerve diameter is of less than 0.1mm, then a better prognosis is predicted, nevertheless, if other associated risk factors are found then a poorer outcome would be probable. Meanwhile, if PNI is affecting larger nerves, then they are often accompanied by more offensive factors directing their outcome^[22].

The presence of multiple PNI foci with neural affinity of tumor cells may be attributed to extensive cancer size and stage together with the presence of the tumor in areas such as the tongue and lip that comprise various neurovascular bundles, therefore proposing that the presence of such multifocal foci in a limited tumor size may enhance the possibility of a more neurotropic tumor action^[23].

Approaching tumor depth was found to increase the liability of larger nerve calibers being infiltrated by tumor cells, this may occur in response to increased number of aggressive tumor cells being in contact with these nerves as well as may give the impression of higher amount of biological molecules being released in response to this invasion. This was particularly manifested as the subcategorization of tumor DOI which revealed that the diameter of the largest nerve showed PNI was significantly related to tumor DOI subgroups (mainly those of group B DOI). In defining T3 category in the recent edition of cutaneous SCC, it was mentioned to comprise a deep invasion of tumor that lie deeper in the subcutaneous adipose tissue (usually beyond 6mm) or those tumors that show PNI involving nerves of 0.1mm or more in diameter^[24]. Actually those deep tumors that was defined in this T3 category corresponds to group B DOI in the study sample, and indicates how tumor DOI or PNI are overlapping factors that may define tumor course.

This study also showed that PNI potential can be greatly increased if young and tongue resections are examined. In fact the offensive tumor behavior that often being witnessed with younger individuals may be attributed to increased PNI potential, as well, the rich neurovascular bundles present in the tongue may enhance more neural affinity of tumor cells. This was in line with a previous study assumed that in each case of SCC affecting the tongue, PNI should be expected^[25].

In conclusion, the detection of PNI should always be considered in: advancing tumor depth, while examining young individuals and tongue resected tumors.

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المستخلص:

ا عتبرت اللجنة الأمريكية المشتركة المعنية بالسرطان في كتيبها التدريبي للطبعة الثامنة أن انتشار الخلايا السرطانية المحيط بالعصب هو أحد أهم العوامل النذيرة لسرطان الخلايا الحرشفية للشفة والتجويف الفمي، كما أضافت عمق الورم في تحديد فئة حجم الورم في نظام التدريج السرطاني الجديد. أجريت هذه الدراسة لتقبيم تواتر انتشار الخلايا السرطانية المحيط بالعصب في سرطان الخلايا الحرشفية الفموي وتأثير زيادة عمق الورم في هذه العملية.

المواد وطرائق العمل: تم قطع أربعة وخمسون حالة من سرطان الخلايا الحرشفية الفموي المستاصلة جنريا والمطمورة بشمع البارافين و صبغها بالهيماتوكسلين والايوسين، ومن ثم تقييمها لانتشار الخلايا السرطانية المحيط بالعصب مع تقدير عمق الورم لكل حالة.

النتائج: اظهرت الدراسة اثنين وعشرين حالة من الانتشار السرطاني المحيط بالعصب وكان هناك علاقة ذات دلالة إحصائية بين اكبر قطر للحزمة العصبية التي اظهرت انتشارا سرطانيا مع عمق الورم حيث ان(p=0.025) ، كما اظهرت الدراسة علاقة ذات دلالة احصائية بين حالة الانتشار السرطاني المحيط بالعصب مع عمر المرضى (p=0.037)وكذلك مع موقع الورم(p=0.004) ، في حين لم تظهر الدراسة اي علاقة ذات دلالة احصائية مع مرتبة ومرحلة الورم (p=0.848, p=0.520) على التوالي.

الاستنتاجات: ان الامكانية الهجومية التي تترافق مع زيادة عمق الورم وكذلك الاور ام التي تصيب الافراد الشباب قد تنعكس عبر تسلل الخلايا السرطانية المحيط بالعصب. كما يجب فحص أورام اللسان المصابة بعناية لهذه العملية البيولوجية الخادعة