Worst pattern of invasion and other histopathological features in oral cancer as determinants of prognosis and survival rate: A retrospective cohort analysis

HANI Z. MARZOUKI[1], AFNAN F. BUKHARI[1], DOAA A. AL-GHAMDI[2], REEM M. ABDULLAH[2], MARWAN AL-HAJEILI[3], SHADI KHAYYAT[4], REEM M. ALZAHRANI[5], YARA R. ALOTAIBI[6], ROLINA AL-WASSIA[7], HATIM AL-MARZOUKI[8] and MAZIN MERDAD[1]

[1]Department of Otolaryngology-Head and Neck Surgery, King Abdulaziz University, Jeddah 23624; [2]Department of Anatomical Pathology and Laboratory Medicine, King Fahad Medical City, Riyadh 12231; Departments of [3]Medicine, [4]Internal Medicine and [5]Otolaryngology-Head and Neck Surgery, King Abdulaziz University, Jeddah 23624; [6]Department of Otolaryngology-Head and Neck Surgery, King Fahad General Hospital, Jeddah 23325; Departments of [7]Radiation Oncology and [8]Radiology, King Abdulaziz University, Jeddah 23624, Kingdom of Saudi Arabia

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Abstract. Oral cavity squamous cell carcinoma (OCSCC) is a well-recognized malignancy of the head and neck. Studies on patients with early-stage oral cancer have shown that they develop locally recurring and/or regional lymph node metastasis, which results in disease-associated mortality. Thus, early-stage oral cancer does not always present good prognoses. The present study aimed to determine the efficacy of using worst pattern of invasion (WPOI) and other histopathological features, such as prognostic factors in OCSCC, and analyze the impact of resection margin status and histopathological prognostic indicators on local recurrence (LR) and overall survival (OS) in patients with OCSCC. A retrospective cohort study was conducted by reviewing the charts of 63 patients with OCSCC treated with primary surgery at King Abdulaziz University Hospital between 2012 and 2019. An author and an experienced pathologist reviewed pathology slides. Associations of histopathological factors, including differentiation, stage, lymphovascular invasion, extracapsular extension, perineural invasion (PNI), WPOI and surgical margins, with LR or disease-free survival (DFS) were evaluated. Univariate analysis identified WPOI and PNI, and multivariate analysis identified the WPOI as predictive factors for LR and DFS. Kaplan-Meier analysis identified the WPOI and PNI as predictive factors for OS and WPOI as a predictive factor for DFS. Therefore, it may be concluded that WPOI and PNI are significant independent prognostic factors for local tumor control and DFS in patients with OCSCC.

Correspondence to: Dr Hani Z. Marzouki, Department of Otolaryngology-Head & Neck Surgery, King Abdulaziz University, Al Murtada, Jeddah 23624, Kingdom of Saudi Arabia
E-mail: hanimarzouki@gmail.com

Abbreviations: DOI, depth of invasion; LR, local recurrence; LVI, lymphovascular invasion; PNI, perineural invasion; WPOI, worst pattern of invasion; PPOI, predominant pattern of invasion

Key words: oral cancer, head and neck squamous cell carcinoma, cancer invasion

Introduction

Oral cavity squamous cell carcinoma (OCSCC) is a malignancy that accounts for 2-3% of all malignancies (1-5). In addition, 90% of all oral cancers arising from the oral mucosa are squamous cell carcinoma (SCC) in origin (6,7). The mainstay of the clinical assessment of oral lesions is a histological diagnosis. Treatment decisions should be based on a microscopic diagnosis instead of clinical presentation, since the prediction of which lesions will progress to invasive carcinoma and which will remain stable with an indolent clinical course is challenging.

Numerous variables have been identified as potential prognostic factors in oral carcinoma, and these can be mainly categorized as tumor-, patient- and treatment-related factors (8). The Tumor, Node, Metastasis (TNM) stage, histological grade and tumor thickness are widely recognized as prognostic factors; however, the prognostic value of other clinicopathological factors is often uncertain and controversial (9).

Multiparametric histological risk assessment has been reported to predict the survival of patients and differentiate between high- and low-risk patients. Several parameters have been used to predict the outcome of malignant disease in OCSCC, including lymphovascular invasion (LVI), perineural invasion (PNI), worst pattern of invasion (WPOI), surgical margin depth of invasion (DOI) and extracapsular extension, which are widely used as indicators of aggressive behavior (10-12). These have been reported to be adverse prognostic factors in OCSCC, associated with the risk of local recurrence (LR) and lymph node metastasis (13,14).

The present study aimed to determine the efficacy of WPOI and other histopathological features as prognostic factors for OCSCC and analyze the impact of resection margin status and histopathological as prognostic factors for LR and the overall survival (OS) of patients with OCSCC in a study population.

Materials and methods

Study design. A retrospective cohort study was designed to determine the efficacy of WPOI and other histopathological features as prognostic factors in oral cancer.

Patients and setting. All patients diagnosed with OCSCC, treated and followed up at King Abdulaziz University Hospital (KAUH; Jeddah, Saudi Arabia) between January 2012 and December 2019 were included in the study. All patients who underwent surgical resection of the primary tumor, with or without radiation therapy or chemoradiotherapy were included. A chart review of these patients was conducted to determine the following parameters: Age, sex, risk factors (tobacco and alcohol), lesion site, TNM staging, histopathological parameters, treatment protocol, treatment response and outcome. The medical records of all the included patients were reviewed and carefully studied. Patients who had only a biopsy of the primary tumor with insufficient follow-up data were excluded from the study.

Medical records. A datasheet was created to include all the demographic data of the patients, including medical record number, age at the time of diagnosis, sex and risk factors. The datasheet also included the disease parameters, namely the diagnosis, date of diagnosis and site of the primary lesion. The type of surgical intervention, whether reconstruction was performed, neck dissection, date of the intervention, and the administration of radiation or chemotherapy were also included. In addition, histopathological factors [tumor grade (differentiation), DOI measured from the tumor surface to the deepest point of invasion, TNM stage, lymphatic invasion, blood vessel invasion, WPOI and PNI], regional control, disease-free survival (DFS), date of recurrence, OS and date of death or last follow-up were also recorded.

An author and an experienced pathologist at King Abdulaziz University Hospital performed a histopathological review of the obtained records. After retrospective data collection, all the specimens were re-examined to evaluate and examine the WPOI, which was determined via the assessment of hematoxylin and eosin-stained and pan-cytokeratin-immunostained sections. Differentiation was classified according to the World Health Organization grade (15). Staging was classified according to the eighth edition of the American Joint Commission on Cancer/Union for International Cancer Control TNM classification (16). All information obtained during the study was kept confidential, including the identities of the subjects, who were assigned anonymous identification numbers.

Statistical analysis. Statistical analysis was conducted using IBM SPSS statistics version 20.0 (IBM Corp.). Basic descriptive statistics were used to compare patient characteristics, including socioeconomic, clinical and treatment characteristics (surgery alone vs. surgery with radiation therapy or chemoradiotherapy), histopathological features (tumor size, DOI, WPOI, PNI, LVI, extracapsular extension and surgical margins), tumor characteristics (tumor site, stage and type of histopathological differentiation), patient outcome and OS. Univariate and multivariate binary logistic regression analyses were used to evaluate the effects of specific risk factors on outcome and recurrence rate. Kaplan-Meier survival analysis was used to evaluate the influence on LR, OS and DFS of various factors, including the histopathological pattern of invasion (POI). DFS was analyzed based on the recurrence rate for different WPOI categories.

Results

Patient sociodemographic characteristics. A total of 63 patients were included in the study, of which 34 (54%) were men and 29 were women, with a median age of 61 years (range, 31-87 years). Patient age was categorized into two groups: ≤60 years and >60 years. The median follow-up period was 473 days (range, 3-2,422 days). Eight patients (12.7%) were smokers, 4 patients had never smoked and the smoking status of the remainder of the patients (n=51; 81%) was unknown. All patients denied a history of current or previous alcohol consumption.

Clinical characteristics. Of the 63 patients, 61.9% had SCC of the tongue (n=39), 27% of the buccal mucosa (n=17), 3.2% each of the hard palate (n=2), inferior alveolar ridge (n=2) and floor of the mouth (n=2) and 1.6% of the superior alveolar ridge (n=1). According to the TNM staging classification, 15 (23.8%), 16 (25.4%), 4 (6.3%), 23 (36.5%) and 5 (7.9%) patients were classified as having TI, T2, T3, T4A and T4B disease, respectively. Concerning lymph node staging, a high proportion of the patients (41.3%; n=26) had no regional lymph node involvement, but 4 (6.3%), 17 (27%), 11 (17.5%), 3 (4.8%) and 2 (3.2%) patients were classified as having N1, N2a, N2b, N2c and N3a disease, respectively. Among the different treatment modalities, 11 (17.5%) of patients received surgery alone, 52 (82.5%) underwent surgery followed by adjuvant radiotherapy and 16 (25%) underwent surgery with adjuvant chemoradiotherapy. Overall, 41.3% of patients experienced tumor recurrence, while 58.7% had no recurrence, were lost to follow-up or did not develop the outcome by the end of the study. Death was reported for 18 patients, and the others were alive, lost to follow-up or had an undocumented death status.

WPOI. In regard to the WPOI, the most frequently observed classification was grade 3 in 24 patients (38.1%), and 10 (15.9%), 6 (9.5%), 13 (20.6%) and 10 (15.9%) cases were classified as grades 1, 2, 4 and 4, respectively. WPOI was re-categorized into 'aggressive' and 'non-aggressive' patterns, where aggressive included grade 4 and 5 tumors and non-aggressive included grade 1-3 tumors.

Other histopathological factors. PNI was observed in 49.2% of the patients. The relationship of PNI to the tumor was intramural in 62.9% and intratemporal in 37% of cases. Regarding the size of the nerve, of the 31 cases with PNI, 9 tumors affected large nerves and 18 tumors affected small nerves. The remaining cases could not be categorized due to lack of documentation. LVI was detected in only 19% of the patients. Regarding the surgical

Table I. Univariate logistic regression analysis of risk factors affecting recurrence rate.

Risk factor	Recurrence		Odds ratio	Nagelkerke R^2	P-value
	No	Yes			
Age (years)					
≤60	20	15	(Ref.)		
>60	17	11	0.863	0.002	0.775
Histopathological differentiation grade					
Well-differentiated	18	13	(Ref.)		0.994
Moderately differentiated	16	11	0.952		0.927
Poorly differentiated	3	2	0.923	0.000	0.935
Staging					
Early-stage oral cancer pattern	19	12	(Ref.)		
Advanced stage oral cancer pattern	18	14	1.231	0.004	0.685
DOI (mm)					
≤5	14	7	(Ref.)		0.659
>5 and ≤10	9	7	1.556		0.518
>10	14	12	1.714	0.018	0.375
WPOI					
Non-aggressive pattern	36	4	(Ref.)		
Aggressive pattern	1	22	198.000	0.749	<0.0005
LVI					
Absent	32	19	(Ref.)		
Present	5	7	2.358	0.037	0.189
PNI					
Absent	30	2	(Ref.)		
Present	7	24	51.429	0.602	<0.0005
Surgical margins					
Free (≥5 mm)	35	20	(Ref.)		0.388
Close (<5 mm)	2	4	3.500	0.116	0.169
Positive	0	2			0.999

DOI, depth of invasion; LVI, lymphovascular invasion; PNI, perineural invasion; WPOI, worst pattern of invasion.

margins, most of the patients had a tumor-free surgical margin (87.3%; n=55), 9.5% (n=6) had close margins and only 3.2% (n=2) had positive margins. According to the histopathological differentiation of SCC, well-differentiated SCC was the most reported type (49.2%). By comparison, poorly differentiated SCC was the least reported type (7.9%) and moderately differentiated was of intermediate incidence (42.9%). Comparing the histopathological differentiation of recurrent cases, among the 31 cases diagnosed as well-differentiated 13 (41.9%) were recurrent, and among the 27 cases diagnosed as moderately differentiated and the 5 cases diagnosed as poorly differentiated, 11 (40.7%) and 2 (40.0%), respectively, were recurrent. Tumor size was categorized according to the T staging; in the majority of the patients (54%) the tumor size was >2 cm but ≤4 cm. Regarding the DOI, one-third (33.3%) of SCCs were ≤5 mm in depth, 25.4 % were between 5 and 10 mm in depth, and 41.3% were >10 mm in depth. Extracapsular extension was present in 18 patients (28.6%).

Univariate analysis. Univariate logistic regression analysis was used to evaluate which risk factors, if any, influenced the recurrence rate of OCSCC. No significant association was detected with age, histopathological differentiation, T stage, DOI, LVI or surgical margin. However, a significant influence of WPOI [74.9%, odds ratio (OR)=198, P<0.0005] was observed, with recurrence significantly more likely in subjects with the aggressive pattern than in those with the non-aggressive pattern. Patients with PNI were also significantly more likely to experience recurrence (60.2%, OR=51.429, P<0.0005; Table I).

Univariate logistic regression analysis was also used to evaluate which risk factors, if any, influenced OS. No significant association with age, staging, WPOI or PNI was detected. However, a significant effect of the presence of LVI (12.8%, OR=5.091, P=0.016) and margins (10.6%, OR=1.060, P=0.043) was observed when comparing close (<5 mm) with free (≥5 mm). Other factors identified as being significant influences on OS included DOI (14.5%, OR=5.029, P=0.026)

Table II. Univariate logistic regression analysis of risk factors affecting death status.

	Death				
Risk factor	No	Yes	Odds ratio	Nagelkerke R^2	P-value
Age (years)					
≤60	27	8	(Ref.)		
>60	18	10	1.875	0.028	0.265
Histopathological differentiation grade					
Well-differentiated	25	6	(Ref.)		0.064
Moderately differentiated	19	8	(Ref.)		0.365
Poorly differentiated	1	4	16.667	0.153	0.020
Staging					
Early-stage oral cancer pattern	25	6	(Ref.)		
Advanced stage oral cancer pattern	20	12	2.500	0.057	0.116
DOI (mm)					
≤5	19	2	7.389		0.077
>5 and ≤10	9	7	5.029	0.145	0.026
>10	17	9	(Ref.)		0.057
WPOI					
Non-aggressive pattern	32	8	(Ref.)		
Aggressive pattern	13	10	3.077	0.085	0.052
LVI					
Absent	40	11	(Ref.)		
Present	5	7	5.091	0.128	0.016
PNI					
Absent	26	6	(Ref.)		
Present	19	12	2.737	0.069	0.085
Size of the nerve					
Small	14	4	(Ref.)		
Large	3	6	7.000	0.234	0.032
Surgical margins					
Free (≥5 mm)	42	13	(Ref.)		0.104
Close (<5 mm)	2	4	1.060	0.106	0.043
Positive	1	1	3.231		0.418

DOI, depth of invasion; LVI, lymphovascular invasion; PNI, perineural invasion; WPOI, worst pattern of invasion.

when comparing 6-10 mm with ≤5 mm, histopathological grade (15.3%, OR=16.667, P=0.020) when comparing poorly differentiated to well-differentiated, and size of the nerve (23.4%, OR=7.000, P=0.032) when comparing large (>1 mm) to small (<1 mm; Table II)

Multivariate analysis. Multivariate binary logistic regression analysis was used to test the risk factors for recurrence. Only two variables were entered into the model, namely WPOI and PNI, as they were the only variables with P<0.1 in the univariate analysis of LR. As a result, the model was accurate (R^2=0.768). However, only WPOI was found to be a significant risk factor with P=0.001 (OR=66), indicating that those with the aggressive pattern were more likely to experience a recurrence (Table III).

Multivariate binary logistic regression analysis was also used to test the risk factors for mortality. The variables shown in Table IV were entered into the model, which were the variables with P<0.1 in the univariate analysis. The model was accurate (R^2=0.614), but only the size of the nerve was found to have a significant effect (OR=26.364, P=0.038) when comparing large (>1 mm) with small (<1 mm).

Survival analysis

OS. OS was calculated from the time (in days) of initial surgical management to the date of the event (death), or to the censoring time in patients who did not develop the event by the end of the study or whose death status was not reported.

Kaplan-Meier estimates were calculated according to the stage of oral cancer by creating two groups: Those with

Table III. Multivariate binary logistic regression analysis of risk factors affecting the recurrence rate.

			95% CI	
Risk factor	P-value	Odds ratio	Lower	Upper
WPOI (non-aggressive vs. aggressive)	0.001	66.000	5.079	857.679
PNI (absent vs. present)	0.142	5.000	0.584	42.797

CI, confidence interval; PNI, perineural invasion; WPOI, worst pattern of invasion.

Table IV. Multivariate binary logistic regression analysis of risk factors affecting mortality status.

			95% CI	
Risk factor	P-value	Odds ratio	Lower	Upper
Age	0.455	3.566	0.452	54.453
Grade	0.210	5.321	0.391	72.455
Staging	0.999	0.000	0.000	33.478
DOI	0.688	2.685	0.432	60.486
WPOI	0.709	2.151	0.039	119.432
LVI	0.707	0.496	0.013	19.124
PNI	0.547	2.185	0.172	27.780
Size of the nerve	0.038	26.364	1.198	580.189
Margin	0.865	0.000	0.000	

CI, confidence interval; DOI, depth of invasion; LVI, lymphovascular invasion; PNI, perineural invasion; WPOI, worst pattern of invasion.

stages I and II (early stage) and those with stage III, IVa, and IVb (advanced stage) oral cancer. According to the Kaplan-Meier survival estimates for the entire cohort, assuming a 0.05 level of significance and using the log-rank test for equality of survivor functions, a statistically significant difference in time-to-death was observed between the groups (P<0.0005; Fig. 1).

Kaplan-Meier estimates were also calculated according to WPOI by creating two new groups: Those with grade 4 and 5 tumors (aggressive pattern) and those with grade 1-3 tumors (non-aggressive pattern). According to the Kaplan-Meier survival estimates for the entire cohort, assuming a 0.05 level of significance and using the log-rank test for equality of survivor functions, a statistically significant difference in time-to-death was detected between the groups (P=0.036; data not shown). Furthermore, when focusing on the patients with early-stage oral cancer, a statistically significant difference in time-to-death between the aggressive and non-aggressive groups was identified (P<0.0005; Fig. 2). However, no significant difference was found between the aggressive and non-aggressive groups for the patients with advanced-stage oral cancer (P=0.679; Fig. 3).

Kaplan-Meier estimates were calculated for OS in relation to PNI by creating two groups: Those with PNI and those without. According to the Kaplan-Meier survival estimates for the entire cohort, assuming a 0.05 level of significance and using the log-rank test for equality of survivor functions, a statistically significant difference in time-to-death was detected between the independent PNI groups (P=0.027; Fig. 4).

DFS. DFS was calculated from the time (in days) of initial surgical management to the date of the event (time to the last follow-up, in years), or to the censoring time of patients who did not develop an event by the end of the study or whose follow-up notes were not available. The same WPOI groups (aggressive and non-aggressive) were used when calculating DFS.

According to the Kaplan-Meier survival estimates for the entire cohort, assuming a 0.05 level of significance and using the log-rank test for equality of survivor functions, a statistically significant difference was identified in time to last follow-up between the aggressive and non-aggressive groups (P<0.0001; Fig. 5). In addition, a statistically significant difference in time to last follow-up between the aggressive and non-aggressive groups was also detected in patients with early-stage oral cancer (P<0.0001; Fig. 6) and those with advanced-stage oral cancer (P=0.002; Fig. 7).

Discussion

The global prevalence of oral malignant disorders is estimated to range from 1 to 5% (8), although much higher rates have been reported in Southeast Asia (17). Nearly 274,300 new oral cancer cases occur worldwide each year (18). It has been shown that the tongue is the most common intraoral site for cancer. SCC constitutes the vast majority (95%) of lingual malignancies and is also the most prevalent type of cancer at other oral sites (19).

Surgery alone is the usual treatment modality for patients with early-stage oral SCC. Unfortunately, LR and/or regional lymph node metastasis develop in certain patients, and disease-related mortality may also occur. However, the prognosis of the disease depends on numerous factors. A multi-parametric histological risk model assessment was initially proposed in 2005, which was reported to predict survival and differentiate between high- and low-risk patients and was validated in a different patient cohort in 2010 (13). This risk model is a modified extension of prior multivariable histological models (20-23). The current study tested the hypothesis that a risk model has prognostic value for early and advanced stage oral cancer patients.

The current study evaluated the efficacy of different histopathological parameters in predicting the outcome of patients with OCSCC. The patients were grouped into a high-risk category (advanced stage oral cancer, stage III-IVb) that would benefit from multimodal treatments, and a low-risk category (early-stage oral cancer, stages I and II) in which local surgical treatment would be adequate.

A well-established association between cancers of the oral cavity and tobacco use has been studied in the literature. In

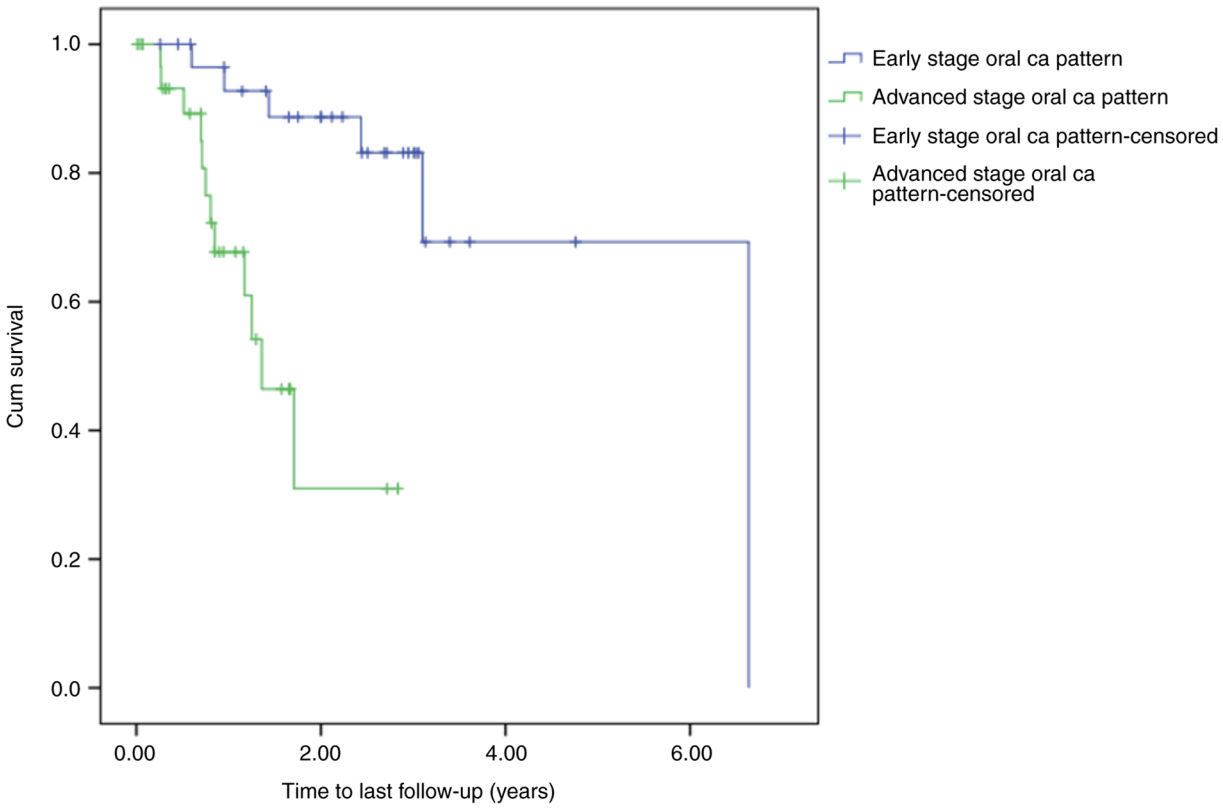

Figure 1. Kaplan-Meier survival estimates for overall survival according to early or advanced stage in the entire cohort. Cum, cumulative; ca, cancer.

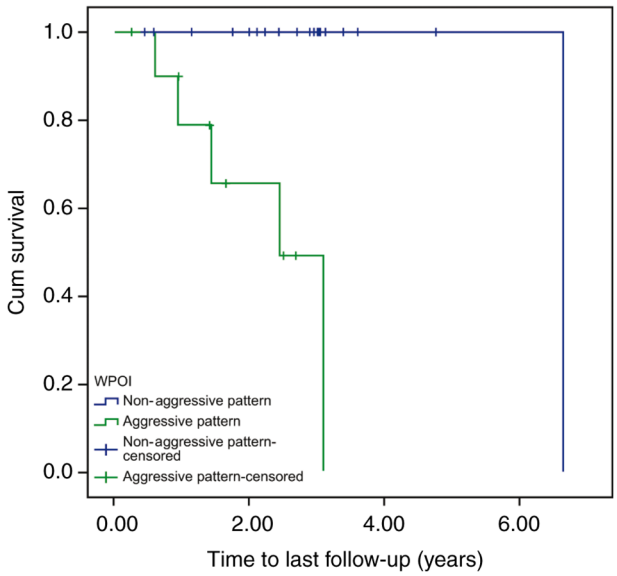

Figure 2. Kaplan-Meier survival estimates for overall survival according to the WPOI in early-stage oral cancer. Cum, cumulative; WPOI, worst pattern of invasion.

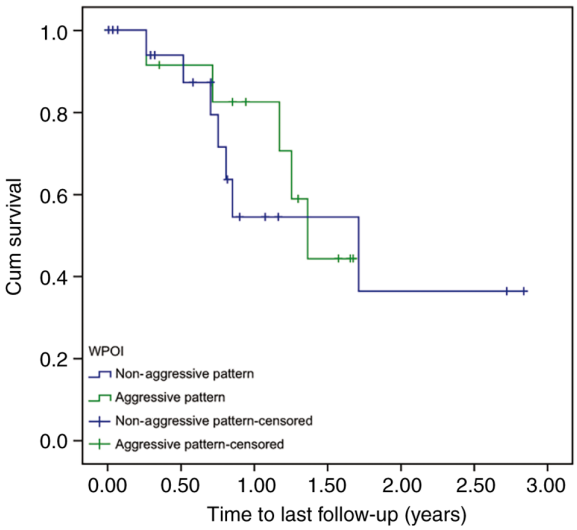

Figure 3. Kaplan-Meier survival estimates for overall survival according to the WPOI in advanced stage oral cancer. Cum, cumulative; WPOI, worst pattern of invasion.

the present study, no association between smoking status and oral cancer recurrence was observed, and no effect of smoking on survival rate was detected (data not shown). These findings can be explained by the smoking status of most patients being unknown.

Alcohol intake has been identified as a significant risk factor for cancers of the aerodigestive tract. In studies where smoking has been controlled for, moderate-to-heavy drinkers have been shown to have a 3-9-fold increased risk of developing oral cancer (24-27). However, none of the patients in the present study admitted to drinking alcohol.

POI was first described by Anneroth *et al* (21) in 1987, who recommended that the tumor structure should be considered as a separate parameter from the tumor cell population. The infiltrative characteristics of the tumor were proposed to be expressed through the POI, categorized into four grades:

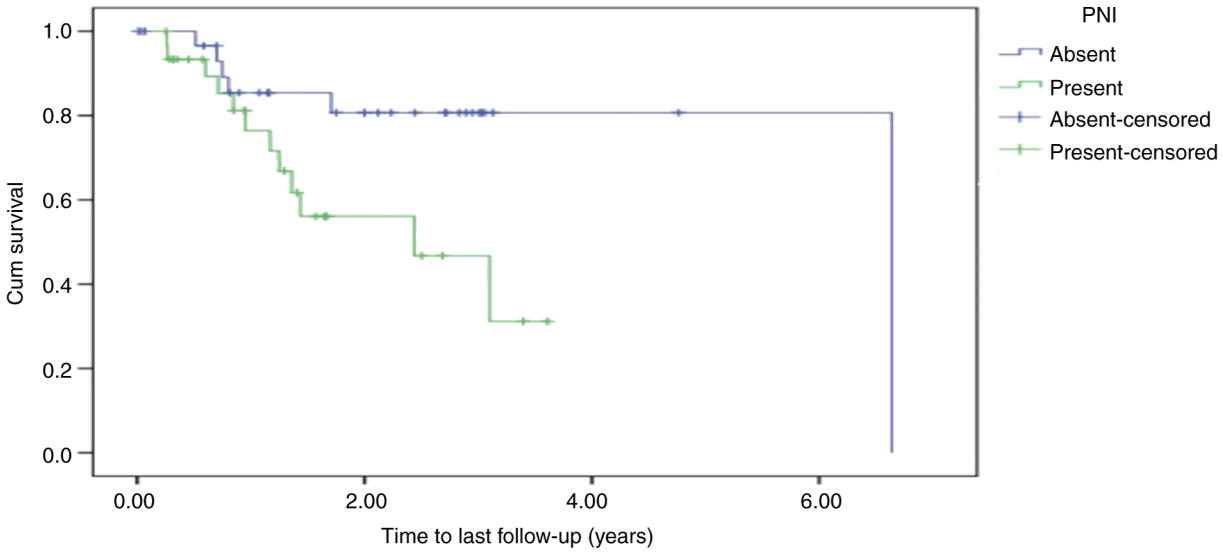

Figure 4. Kaplan-Meier survival estimates for the entire cohort according to the presence or absence of PNI. Cum, cumulative. PNI, perineural invasion.

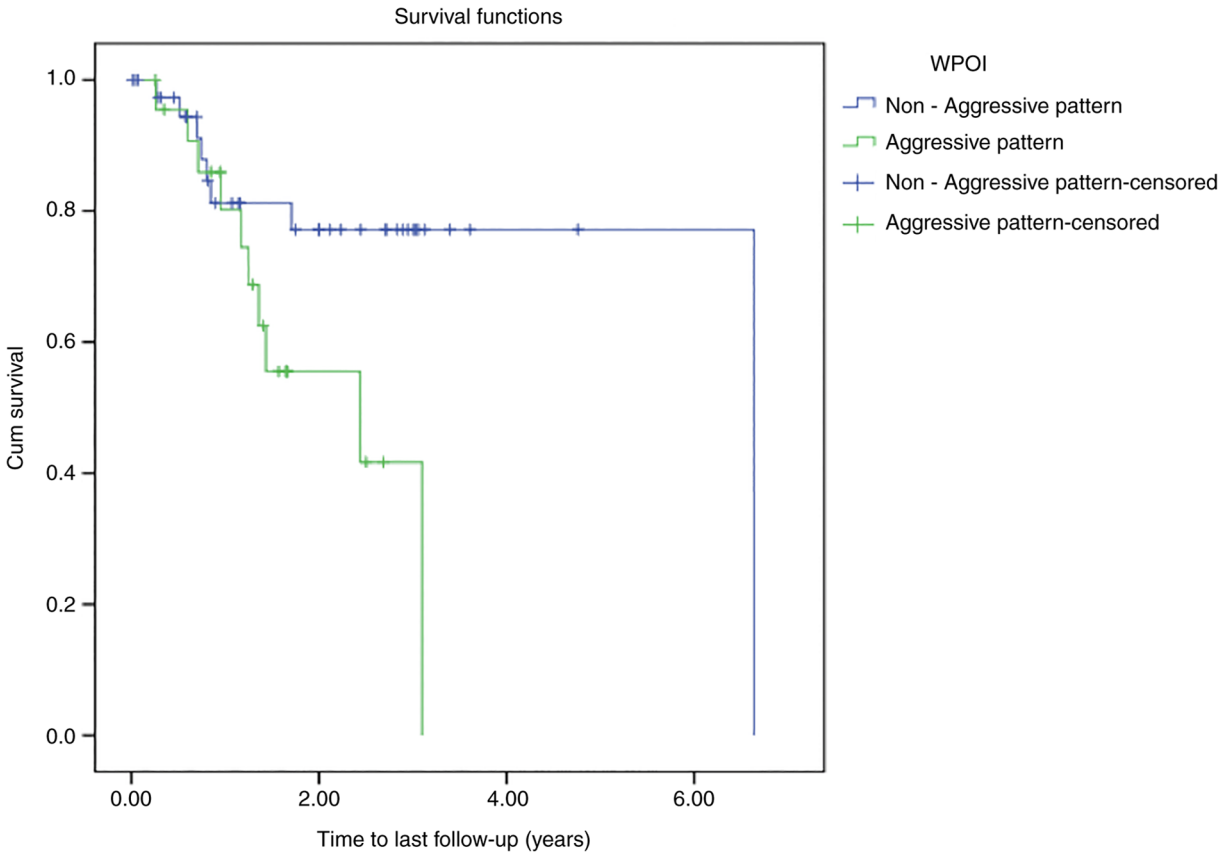

Figure 5. Kaplan-Meier survival estimates for disease-free survival according to the WPOI in the entire cohort. Cum, cumulative; WPOI, worst pattern of invasion.

Grade 1, neoplasm with pressing, well-defined infiltrating border; grade 2, invasion by solid cords and strands of neoplastic cells; grade 3, invasion by small groups of cells or cords (n>15); and grade 4, broad front invasion by single cells or small groups of cells (n<15) (19).

A retrospective study by Bryne *et al* (22) compared Broders' grading method with a modified version of the malignancy grading system proposed by Anneroth *et al* (21) where the latter was performed only within the histologically most invasive tumor areas. Using Cox's multivariate survival analyses, this grading of the invasive sites was found to be of significant prognostic value. On this basis, it was hypothesized that the histologically invasive areas are essentially responsible for the clinical behavior of the tumor, which may be of relevance when

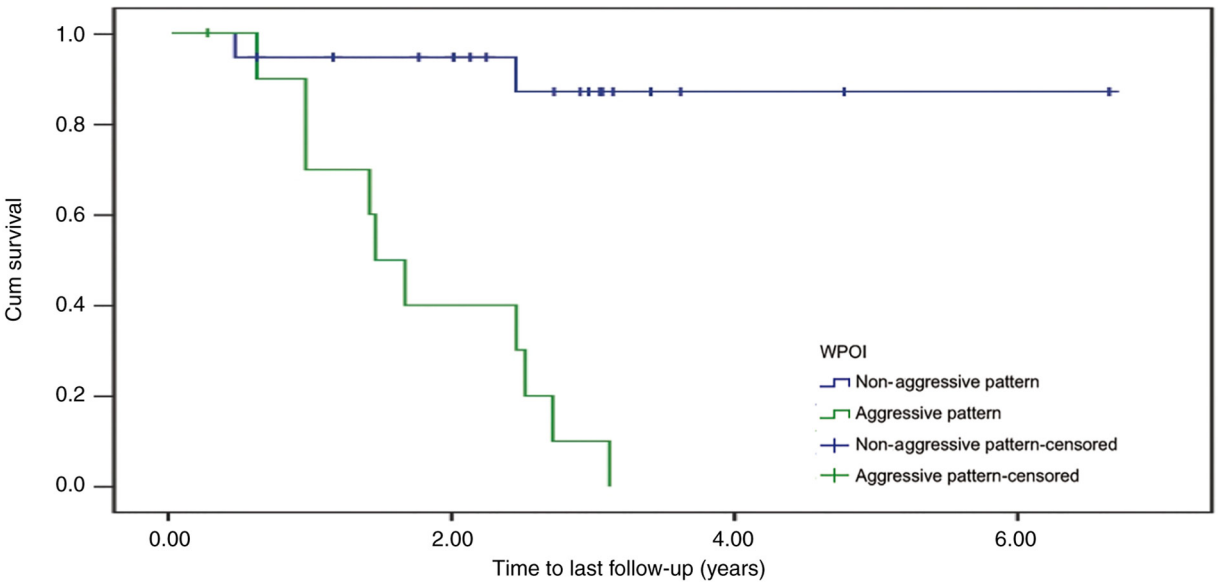

Figure 6. Kaplan-Meier survival estimates for disease-free survival according to the WPOI in early-stage oral cancer. Cum, cumulative; WPOI, worst pattern of invasion.

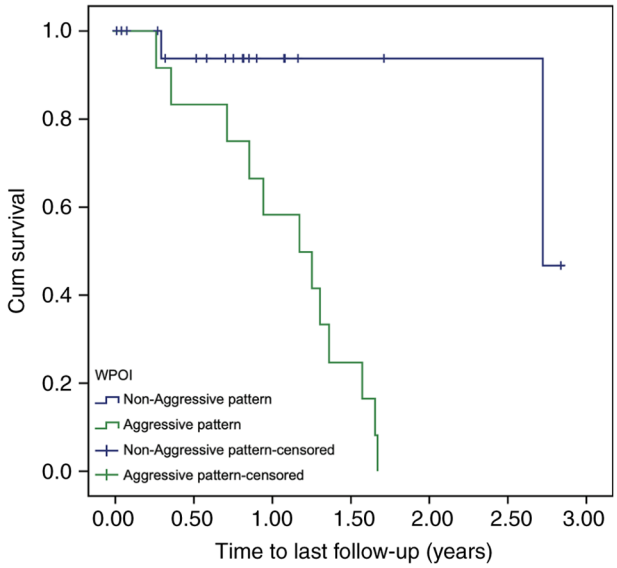

Figure 7. Kaplan-Meier survival estimates for disease-free survival according to the WPOI in advanced-stage oral cancer. Cum, cumulative; WPOI, worst pattern of invasion.

selecting the therapy for OCSCC. Bryne *et al* (22) described the grading system in terms of five morphological features: Degree of keratinization, nuclear polymorphism, number of mitoses, mode of invasion and plasma-lymphocytic infiltration, each scored from 1 to 4 according to definitions proposed by Anneroth *et al* (21). In this system, only cells at the deep invasive margins of the tumors are graded, and the scores for each morphological feature are added to yield a total malignancy score.

In another study by Bryne *et al* (23), all 96 cases of SCCs in the floor of the mouth registered with the Cancer Registry of Norway between 1963 and 1972 were retrospectively analyzed. The study concluded that invasive cell grading is potentially valuable for planning the treatment of oral cancers, and suggested that the deep, invasive parts of oral and other cancers require further study to improve the understanding of tumor cell invasion and metastasis.

Regarding the predictive value of WPOI at the tumor interface, Brandwein-Gensler *et al* (28) conducted a study to examine the effect of surgical margin status and histological prognosticators on LR and OS for patients with OCSCC. The traditional Bryne WPOI was expanded by the addition of pattern 5, defined as tumor satellites (regardless of size) dispersed ≥1 mm from the closest intervening tumor island. In this study, Brandwein-Gensler described POIs as: Grade 1, pushing border; grade 2, finger-like growth; grade 3, large separate islands, >15 cells/island; grade 4, small tumor islands, ≤15 cells/island; and grade 5, tumor satellites, ≥1 mm from the main tumor or next closest satellite. They also validated the process of considering only the WPOI by comparing predominant POI (PPOI) with the WPOI at the tumor/host interface. WPOI 4 and WPOI 5 were found to be high-risk patterns significantly associated with OS compared with WPOI 1-3 (28).

In the present study, WPOI was categorized into aggressive and non-aggressive patterns, where aggressive included grades 4 and 5, and non-aggressive included grades 1-3. Comparative univariate analysis showed a significant association between aggressive patterns and recurrence rate. The WPOI effect was 74.9% (OR=198, P<0.0005) when the aggressive pattern was compared with the non-aggressive pattern. Multivariate binary logistic regression analysis modeling indicated that WPOI was significantly associated with LR (P=0.001). The model was significant (R^2=0.768, OR=66) when the aggressive pattern was compared with the non-aggressive pattern; thus, patients with an aggressive pattern were more likely to develop recurrence.

In North India, a retrospective validation study of the Brandwein-Gensler risk model in 149 patients with OCSCC conducted by Chaturvedi *et al* (29) showed that aggressive type WPOI was significantly associated with LR (P=0.016).

Almangush *et al* (30) conducted a retrospective study in 479 patients from three countries, who were treated for early-stage OCSCC between 1979 and 2012. Comparing the invasive pattern to the cohesive pattern, the authors found that in early-stage OCSCC, WPOI was a strong pathological predictor for locoregional recurrence and death.

With regard to WPOI and survival, the aforementioned study conducted by Brandwein-Gensler *et al* (28) used Cox regression analyses to examine the effect of surgical margin status and histological prognostic indicators on LR and OS for patients with OCSCC. The study concluded that WPOI 4 (P=0.004) and 5 (P=0.001) tumor types were significantly associated with OS in comparison with WPOI 1-3 tumor types. In addition, as WPOI and PPOI were both predictive of OS, the authors suggested that it is valid to use WPOI as a variable in place of PPOI since WPOI was found to associated with LR, but PPOI was not.

A study that included only patients with stage I disease was performed by Bundgaard *et al* (31) with the aim of confirming that not all histological parameters are equally important predictors of malignancy. A total of 78 patients with stage I (T1N0M0) oral SCC from two different ear, nose and throat departments were included in the study. POI was found to be the only significant prognostic parameter for disease-specific survival (P=0.04). Hori *et al* (32) conducted a retrospective study of 62 patients with early-stage OCSCC, defining grades 4 and 5 as the WPOI. Univariate analysis identified WPOI (P<0.001) to be a predictive factor for DFS, and multivariate analysis identified WPOI (hazard ratio=3.84, 95% CI=1.30-11.34, P<0.05) to be an independent histopathological risk factor for DFS.

A retrospective study of 340 patients with early-stage tongue SCC evaluated various histopathological prognostic indicators. In the study, WPOI was divided into a two-tiered system in which score 0, representing grades 1-3, was considered low and scores 1 and 3, representing grades 4 and 5, respectively, were considered high, and a statistically significant association of WPOI with mortality from oral tongue SCC was identified. The patients with a high WPOI score (defined as <15 cells in an invasive island, single cells, or satellite tumor cells) were associated with higher mortality compared to those with a low WPOI score (defined as pushing borders, finger-like and cohesive invasion) (30).

In the current study, other factors that could be relevant to LR and DFS were evaluated, including age, sex, PNI, surgical margin status, LVI, T and N stage, DOI and histopathological differentiation. Patient age was categorized into two groups, ≤60 and >60 years, with a median age of 61 years (range, 31-87 years). The results of the present study did not show any influence of age on prognosis. Whilst the disease itself was more commonly found in males, the sex of a patient was not observed to have a significant effect on the LR. In univariate analyses, age and sex did not significantly influence LR or DFS.

PNI is a well-recognized prognostic factor for survival and LR. In the present study, univariate logistic regression analysis was used to test if the presence of PNI affected the recurrence rate. The effect of PNI was 60.2% (OR=51.429, P<0.0005) when the presence and absence of PNI were compared. Kaplan-Meier estimates were calculated in patients with and without PNI. The Kaplan-Meier survival analysis of the entire cohort revealed a statistically significant difference in time-to-death between the independent PNI groups (P=0.027). In a retrospective study on patients with OCSCC conducted by Chaturvedi *et al* (29), 53 of the 149 specimens included in the study (35.5%) were found to have PNI. Additionally, PNI was observed in 10 of the 17 patients with recurrence (58.8%) and exhibited a statistically significant association with recurrence (P=0.03). However, it was not found to be associated with disease-specific survival (P=0.39).

The status of the surgical margins was not predictive of LR or survival rates in the present study. However, a strong association between disease-free margin and higher survival rates, with delayed time to recurrence was shown in studies by Guerra *et al* (33) and Woolgar *et al* (34). The results of the present study are consistent with those of Brandwein-Gensler *et al* (28), which found no association between margin status and LR (P=0.2) or OS (P=0.8). Previous studies have found LVI to be highly prognostic; Jones *et al* (35) found a significant association between LVI and survival (P=0.015), while Liu *et al* (36) found LVI to be an independent predictor of DFS. In the current study, LVI was present in only 19% of the patients and was not found to be associated with recurrence or disease-specific mortality in the multivariate analysis.

The present study showed no statistically significant association of the T stage with recurrence rate. The recurrence rate was not found to be affected by T staging (P=0.685). However, a statistically significant association between early-stage and DFS was identified, which may be explained by patients with advanced-stage cancer being lost to follow-up or dying before experiencing a recurrence. N stage did not demonstrate an influence on LR or DFS.

With regard to the effect of histological differentiation on prognosis, differentiation was not found to influence either the risk of recurrence or the DFS in the present study, probably as most of the cases were of well-differentiated SCC.

There were several limitations to the present study. First, because the sample size was small, stratifying various oral cancer stages with no appropriate control group of data could have resulted in misleading associations. In future studies, this can be minimized by controlling or matching factors that could produce such associations, especially with the differences in the treatment modalities used, risk factors, recurrence rate, and OS. A larger sample size with a larger number of events may produce different conclusions. Second, given the retrospective nature of the study, it may be subject to selection bias, since 18 patients were excluded from the analysis due to incomplete data. Third, again because this study was retrospective, interpretation of the data is highly dependent on what was documented at the time of surgery and at the time of follow-up in the outpatient clinic. Finally, the study was performed in a single center and, therefore, the results require external validation to support widespread changes in practice.

In conclusion, according to the data in the present study, those patients with OCSCC who had an aggressive POI or the presence of PNI had worse clinical outcomes. Moreover, WPOI and PNI were found to be significant independent prognostic indicators for local tumor control and DFS. Therefore, follow-up plans for patients, especially those with early-stage OCSCC, should consider these pathological invasion patterns on surgical specimens. In addition, multimodal treatment is likely to benefit patients with early-stage oral SCC in whom

aggressive high-risk disease is found by evaluating these factors. Based on the present findings, a multicentric analysis of pooled data is recommended for better clarity on this issue.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HZM was the primary contributor to manuscript preparation, created and designed the study, made major contributions to writing the manuscript and revised the manuscript. AFB analyzed the data, made major contributions to writing the manuscript, and was responsible for all other aspects of the submission, including data collection, interpretation of data, analysis and manuscript preparation. RMAl and YRA collected the data by reviewing the patient hospital records, prepared the manuscript and reviewed the literature. DAA and RMAb assessed histopathology slides and reviewed the literature. MAH and SK verified the analytical methods and reviewed the results. RAW, MM and HAM aided the interpretation of the results and worked on the manuscript. All authors were involved in manuscript preparation. HZM and AFB confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval

The study protocol was reviewed and approved by an institutional review board committee at KAUH, Jeddah, Saudi Arabia. Ethical approval for this study was obtained from the Research Ethics Committee at KAUH (ref. no. 662-19).

Patient consent for publication

Informed consent for publication was waived due to the retrospective nature of the study.

Competing interests

The authors declare that they have no competing interests.

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