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The predictive role of tumor infiltrating lymphocytes and pathohistological parameters for the occurrence of metastases in the clinical N0 neck of early-stage oral squamous cell carcinoma

Предиктивна улога тумор инфилтришућих лимфоцита и патохистолошких параметара за појаву метастаза на клиничком *N*0 врату раног стадијума оралног сквамоцелуларног карцинома

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Предиктивна улога тумор инфилтришућих лимфоцита и патохистолошких параметара за појаву метастаза на клиничком *N*0 врату раног стадијума оралног сквамоцелуларног карцинома

SUMMARY

Introduction/Objective With 337,173 registered cases in the world in 2020, oral squamous cell carcinoma is the most common malignant tumor of the head and neck region. The status of lymph nodes in the neck is the most important isolated prognostic factor for the five-year survival of patients. This study aimed to determine the pathohistological predictors of the occurrence of occult neck metastases of early-stage oral squamous cell carcinoma.

Methods The study included 40 patients, aged 62.8 ± 10.7, with early-stage oral squamous cell carcinoma with clinical N0 findings in the neck. All patients underwent radical transoral tumor ablation and elective neck lymphadenectomy. Based on pathohistological findings, the patients were divided into two groups – the group with occult metastases present and the group without occult metastases in the neck.

Results Occult metastases were present in 13 patients included in the study (32.5%). The results of the study indicate a significant difference in parameters regarding desmoplastic reaction of the stroma ($p < 0.001$), depth of invasion ($p < 0.001$), lymphocytic infiltration ($p < 0.001$), and lymphovascular invasion in univariate (OR 24.0, $p = 0.004$) and multivariate (OR 32.713, $p = 0.017$) logistic regression analysis in the group of patients with occult metastases compared to the group of patients without metastases.

Conclusion The values of pathohistological predictors indicate a high degree of correlation with the appearance of occult metastases in the neck. Significance in prediction represents a solid argument for the mentioned parameters to be part of the standard pathohistological examination of oral squamous cell carcinoma.

Keywords: oral squamous cell carcinoma; occult metastases; pathohistological analysis; surgical treatment; observation

САЖЕТАК

Увод/Циљ Орални сквамоцелуларни карцином, са 337.713 регистрованих случајева у свету 2020.године, је најучесталији малигни тумор регије главе и врата. Статус лимфних нодуса врата представља најважнији изоловани прогностички фактор за петогодишње преживљавање пацијената. Циљ овог истраживања је утврдити да ли се на основу патохистолошке анализе препарата раног стадијума оралног сквамоцелуларног карцинома може предвидети појава окултних метастаза на врату.

Методе У студију је укључено 40 пацијената са клиничким *N*0 налазу на врату раног стадијума оралног сквамоцелуларног карцинома. Свим пацијентима је учињена радикална трансорална аблација тумора и елективна лимфаденектомија врата. На основу дефинитивног патохистолошког налаза пацијенти су подељени у групу са присуством и одсуством окултних метастаза.

Резултати У испитиваној групи окултне метастазе су регистроване код 13 пацијената(32,5%). Резултати студије указују на постојање значајне разлике параметара дезмопластичне реакције строме ($p < 0,001$), дубине инвазије ($p < 0,001$), лимфоцитарне инфилтрације ($p < 0,001$) и лимфоваскуларне инвазије у униваријалној (OR 24, $p = 0,004$) и мултиваријалној (OR 32,713, $p = 0,017$) логистичној регресионој анализи у групи пацијената са окултним метастазама у односу на групу пацијената без метастаза.

Закључак Резултати наше студије указују на директну повезаност појаве окултних метастаза на врату и појединих патохистолошких параметара. Сигнификантност у предикцији појаве окултних метастаза представља чврст аргумент да поменути параметри требају бити стандардни део патохистолошког испитивања оралног сквамоцелуларног карцинома.

Кључне речи: орални сквамоцелуларни карцином; окултне метастазе; патохистолошка анализа; хируршко лечење; праћење

INTRODUCTION

With 337,713 registered worldwide cases in 2020, oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the head and neck region [1]. Given that it accounts for up to 90% of the total number of intraoral malignant tumors, it is by far the most common intraoral malignancy [2]. Aggressive behavior towards the surrounding anatomical structures and their rapid infiltration, early appearance of metastases in the neck, and frequent recurrence are some of the key clinical characteristics of OSCC. The five-year survival rate of early-stage OSCC patients varies from 80–90%, contrary to advanced forms characterized by a five-year survival of 30–50% [3]. The fundamentals of OSCC treatment include radical tumor ablation, neck lymphadenectomy, adequate reconstruction of the resulting defect, and postoperative oncological therapy.

The status of lymph nodes in the neck (LNs) represents the most important isolated prognostic factor for the survival of patients with OSCC. The presence of metastases in the neck significantly reduces the five-year survival of patients below 50% [4]. The treatment of N0 neck in the early stages of OSCC (pT1-N0-M0 and pT2-N0-M0) is still the subject of many debates in head and neck surgery without a clearly defined viewpoint for conducting elective neck lymphadenectomy (END) or the so-called watchful-waiting (WW) method, i.e., the observation of patients. The presence of occult metastases (OM) in the values of 16–36%, pathohistologically verified in postoperative dissections of the neck, significantly affects the decrease of the five-year survival of patients [5]. Sentinel lymph node biopsy (SNB) is a routine surgical procedure for the detection of OM in the neck in OSCC. The use of SNB proves high sensitivity for detecting micrometastases and isolated tumor cells (ITC), a lower degree of morbidity of the surrounding tissue, and a considerably more positive economic aspect compared to END [6]. The sensitivity of SNB in the values of 75–87% for registering OM in the early stage of OSCC defines the mentioned method as safe and rational [7].

Literature data suggest that based on the pathohistological analysis of OSCC preparations, which includes depth of invasion (DOI), desmoplastic stromal reaction (DR), tumor budding (TB), lymphovascular invasion (LV), perineural invasion (PN), and density of tumor infiltrating lymphocytes (TILs), the occurrence of extranodal extension (ENE) of OSCC, which is directly related to the occurrence of metastases in the neck, can be predicted [8, 9].

This study aimed to determine whether the aforementioned pathohistological parameters can be reliable predictors for OM occurrence of OSCC stage pT1-N0-M0 and pT2-N0-M0.

METHODS

The study included 40 consecutive patients of both sexes treated at the Clinic for Maxillofacial Surgery in Niš from 2019 to 2022, in whom the presence of OSCC stage cT1-N0-M0 and cT2-

N0-M0 was determined based on medical history, clinical examination, initial biopsy and additional radiological examinations.

The criteria for excluding patients from the study included patients with higher T stages (cT3-cT4), recurrent OSCC that was in rT1-rT2 stage at the time of examination, radiologically confirmed the presence of secondary deposits (M1 stage), initially inoperable forms of OSCC turned into an operable state after oncological treatment, patients in whom the progression of the disease occurred from the verification of OSCC stage cT1-cT2 to the surgical procedure, and patients in whom, due to general condition or comorbidities, intervention in general endotracheal anesthesia (GETA) was contraindicated.

The analysis of anamnestic data determined age, gender and risk factors in terms of alcohol consumption and tobacco smoking.

The clinical examination determined the precise location of the suspicious lesion in the oral cavity as well as its clinical form (exophytic or endophytic). The clinical examination also determined the Karnofsky score (KPS) of the patients, which ranged 90–100 in all subjects (Figure 1).

After the anamnestic and clinical examination, punch biopsy of the suspicious lesion in the oral cavity was performed for the pathohistological verification of OSCC.

Radiological examinations included multi-slice computerized tomography of the head and neck (MSCT), magnetic resonance imaging of the head and neck (NMR), lung imaging and ultrasound (ECHO) of the neck and abdomen. Radiological examinations determined the precise diameter of the tumor, as well as its relation to the surrounding soft tissue and bone structures.

Surgical treatment of patients included radical transoral tumor ablation and END of neck levels I, II and III (Figure 2).

The pathohistological analysis of postoperative OSCC preparations was done after the fixation of the preparations in a 10% solution of buffered formalin. The micromorphological analysis of histological sections made with the standard hemotoxin-eosin (H&E) method included the analysis of the status of resection margins, analysis of DOI, DR, LVI, PNI, tumor grade, and density of TILs. Immunohistochemical analyses were performed by determining the expression of pan-cytokeratin (pan-CK) (AE1/AE3) in suspicious lymph nodes of postoperative neck dissections to identify occult metastatic changes.

The statistical processing of data involved presentation in the form of arithmetic mean, standard deviation, minimal and maximal values, and ratio in the form of absolute and relative numbers.

Numerical variables were compared using the t-test, whereas categorical characteristics were compared with the Chi-square test or Fisher's exact probability test. Logistic regression analysis was used to assess the relationship between the studied variables and the appearance of occult metastases.

We excluded specific variables (DR, DOI, smoking, lymphocyte infiltration) from our logistic regression model due to perfect separation or multicollinearity, which was identified when these variables perfectly predicted the outcome, leading to model estimation issues such as non-convergence. This decision was made to ensure the stability and reliability of our model's parameter estimates.

The null hypothesis was tested with a significance threshold of $p < 0.05$. Statistical data processing was performed in the SPSS 16.0 software package.

The study was approved by the Ethics Committee of the Dental Clinic in Niš (14/14-2019-4 EO) and the Ethics Committee of the Faculty of Medicine in Niš (12-16502/2-1).

RESULTS

The study included 40 patients (25 male and 15 female). The mean age of the studied population was 62.8 ± 10.7 (min 37 years, max 85 years). The majority of subjects were of male sex (62.5%), older than 55 years (75.0%), smokers (72.5%), who regularly consumed alcohol (55.0%) and had exophytic tumor growth (52.5 %).

There were 13 patients with OM in the studied group of patients (32.5%). There was no statistically significant difference with respect to gender ($p = 0.794$), age ($p = 1.000$), alcohol consumption ($p = 0.312$), tumor growth type ($p = 0.370$) and tumor grade ($p = 0.114$) regarding the presence of OM (Table 1). Smoking was statistically significantly more common in patients with OM ($p = 0.007$).

PNI was present in 62.5% of patients and did not differ significantly in relation to the presence of metastases ($p = 0.663$). LVI was statistically significantly different in relation to the presence of metastases (92.3% *vs.* 33.3%, $p = 0.001$). DR differed statistically significantly in relation to the presence of metastases ($p < 0.001$), as well as DOI ($p < 0.001$). Lymphocytic infiltration was statistically significantly different in relation to the presence of metastases ($p < 0.001$). Statistical analysis does not show the existence of a significant correlation between tumor stage of OSCC (T1–T2) nor the presence of occult metastases on the neck.

The changes were localized on the tongue (42.5%), alveolar processes (20%), floor of the oral cavity (17.5%), hard palate (5%), retromolar triangle (7.5%), buccal mucosa (5%), and soft palate (5%) (Figure 3).

Univariate logistic regression analysis showed that LVI is a statistically significant risk factor for OM (OR 24.0, p = 0.004). Moreover, it remained a statistically significant risk factor in the multivariate model (OR 32.713, p = 0.017) (Table 2).

DISCUSSION

The distribution of OSCC in the oral cavity varies on the global level and is primarily dependent on the geographic location that determines exposure to the most frequent etiological factors. The location of OSCC in the oral cavity is an important prognostic factor, as well as an implication of the patient's surgical treatment. Literary data indicate that the most common site of OSCC in Thailand, India and southeastern Asian countries is the buccal mucosa [10]. Available data from Nigeria and Germany highlight the gingiva of the alveolar processes of the lower and upper jaw as well as the floor of the oral cavity as predilection sites for OSCC [11, 12]. OSCC is most common in the tongue (41.7%), floor of the oral cavity and lips (16.5%, each), gingiva of the alveolar processes of the upper and lower jaw (10.6%), buccal mucosa (6.7%), retromolar triangle (5.6%) and hard and soft palate structures (2.3%) [13].

Alcohol and tobacco consumption are the most important etiological factors associated with the occurrence of OSCC. These factors are present in 70–80% of patients with OSCC [14]. Chemical carcinogens from alcohol and tobacco participate synergistically in generating mutations in tumor suppressor genes and thus promote carcinogenesis.

OSCC is considerably more frequent in males than in females. Literature data suggest that men make up 90–93% of patients with OSCC, contrary to women, who represent only 7–10% of patients [15]. A marked discrepancy in the frequency of patients of both sexes is explained by more frequent exposure to etiological factors in men, in contrast to women. The male-female ratio differs primarily at the regional level, but the percentage of men with OSCC is almost always higher.

OSCC is significantly more common in patients older than 50 [16]. The presented data on age vary regarding the region and the number of patients included in the study.

Pathohistological findings in postoperative neck dissections can be negative, indicating the presence of isolated tumor cells (ITCs), micrometastases (MM) or macrometastases. Negative findings imply reactive cellular changes, sinus histiocytosis and benign cell inclusions. By definition, ITCs are small cell aggregates not larger than 0.2 mm, or non-confluent aggregates not exceeding 200 cells per section. Micrometastases represent individual tumor deposits with a diameter of 0.2 mm to 2 mm, unlike macrometastases, which also represent individual tumor deposits, but larger than 2 mm. Considering that it is impossible to prove the existence of ITCs and MM by standard preparation examination (H&E), pan-cytokeratin (pan-CK) (AE1/AE3) has been introduced as a useful marker for determining the presence of OM. The detected presence of ITCs and MM in the value of 33.3% on preparations that were negative by H&E processing indicates that pan-CK (AE1/AE3) is a reliable marker for the detection of ITCs and MM [17]. DOI was defined as the distance from the normal basal membrane to the deepest point of tumor invasion, is an independent risk factor for the occurrence of OM in lymph nodes of the neck in patients with OSCC. Studying patients with early-stage OSCC, the threshold and increased value of DOI would represent an indicator for END [18]. The association between DOI over 5 mm and 36.25% of occult metastases was presented in a study by Pakistani authors [19]. The study included 80 patients with pathohistologically proven OSCC. The authors concluded that patients with DOI +5 have a relative risk of occult neck nodes metastasis and that there is no significant relationship of occult neck node metastasis with age, gender, risk factors and tumor grade. LVI by definition refers to the presence of tumor cells in the lumen of a blood vessel or cellular adhesion to the vascular wall. The presence of LVI is interpreted as a negative prognostic factor for patients suffering from OSCC. In a study that included 442 patients with OSCC, Abigail E. Moore et al. [20] concluded that LVI is a significant pathohistological parameter for the occurrence of regional metastases. The same authors also reported the absence of correlation of LVI with tumor aggressiveness and local recurrence. Prakash Pandit et al. [21] conducted a study on 462 patients with OSCC and confirmed the link between LV and metastases in the lymph nodes of the neck.

PNI represents the appearance of a tumor in close proximity to a nerve, involving at least 33% of its volume, or the presence of tumor cells within any of the three layers of the nerve sheath. The presence of PNI on pathohistological preparations of OSCC is associated with increased morbidity, poor prognosis and frequent local recurrences, but without a significant impact on the occurrence of regional metastases [22]. DR type is one of the key stromal characteristics of malignant tumors of the gastrointestinal tract [23]. Based on the morphology and arrangement of collagen fibres and myxoid stroma, DR is divided into mature, intermediate and immature. The insight into available literature reveals works of numerous researchers who associate DR with the clinical characteristics of OSCC. In a study that included 308 patients with early-stage

OSCC of the tongue, Alhadi Almangush et al. [24] determined a relationship between the stromal reaction type and patient survival. However, the same authors did not find a relationship between the DR type and the appearance of metastases in the neck. Furthermore, Bittar RF et al. [25] conducted a study that included 157 patients with verified OSCC and a clinical N0 finding in the neck and determined an association between the appearance of OM in the neck and the type of DR. Yuri Noda et al. [8] suggested an association between immature tumor stroma and ENE. The aforementioned authors in a study that included 83 patients with pathohistologically verified OSCC indicate the existence of a significant correlation between immature tumor stroma and ENE. In addition, the results of our study indicate a significant correlation between the occurrence of OM and the immature tumor stroma of OSCC preparations.

TILs are a selected population of lymphocytes, predominantly of the T form, with a highly specific immune response to tumor cells. They represent reliable biomarkers for many solid tumors, including malignant tumors of the head and neck [26]. The method for the standardized assessment of the presence of TILs using the H&E method published by The International Immuno-Oncology Biomarker Working Group is being very effectively applied in daily clinical practice [27]. Literature data indicate that high tumor infiltration by TILs represents a favourable prognostic sign for OSCC and other malignant tumors, in terms of an increased survival rate and less aggressiveness of the tumor [28]. The results of our study, which indicate a relationship between the appearance of OM in the neck, aggressiveness of the tumor and low tumor infiltration by TILs, are in correlation with the results of the aforementioned authors [8, 9, 29, 30].

CONCLUSION

The presence of OM in the 16–36% range in postoperative neck dissections undoubtedly has a bad effect on the five-year survival of OSCC patients, which varies 30–90%, depending on the stage of the tumor and the status of LNs.

The results of our study indicate a high degree of association of the analysed pathohistological parameters with the occurrence of OM in the neck in patients with early-stage OSCC. The significance of predicting the risk of OM is a strong argument for the mentioned parameters to be part of the standard pathohistological examination of OSCC.

We firmly believe that our idea of future inclusion of epigenetic examination of OSCC preparations into standard diagnostics will contribute to early detection of OM and thus an increase in the five-year survival of affected patients.

Conflict of interest: None declared.

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Figure 1. Oral squamous cell carcinoma of the tongue

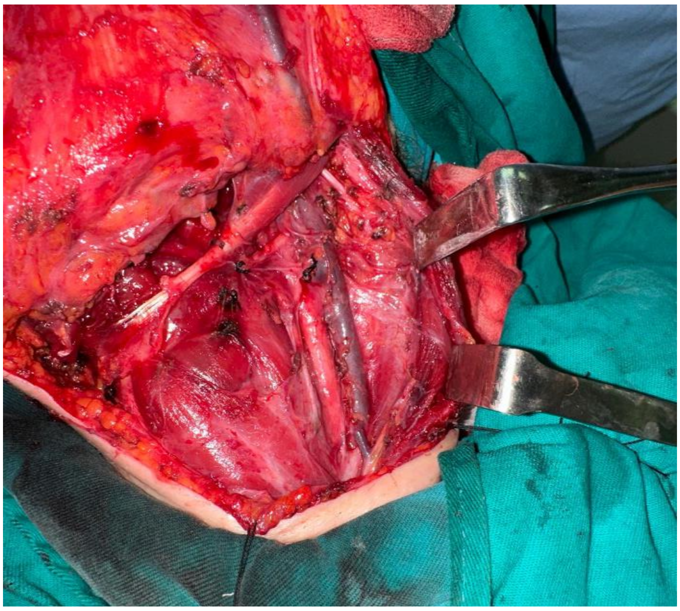

Figure 2. Elective neck lymphadenectomy of levels I, II, and III

Table 1. Demographic and clinical characteristics of patients regarding the presence of metastases

Characteristic		**Total**		**Occult metastases**				**p**[1]
				Yes		**No**		
Sex	male	25	62.5	9	69.2	16	59.3	0.794
	female	15	37.5	4	30.8	11	40.7	
Age (years)	< 55	10	25.0	3	23.1	7	25.9	1.000*
	55+	29	75	10	76.9	20	74.1	
Smoking		29	72.5	13	100	16	59.3	0.007*
Alcohol		22	55	9	69.2	13	48.1	0.312
Growth type	exophytic	21	52.5	5	38.5	16	59.3	0.370
	endophytic	19	47.5	8	61.5	11	40.7	
Tumor grade	I	12	30	1	7.7	11	40.7	0.114*
	II	20	50	9	69.2	11	40.7	
	III	8	20	3	23.1	5	18.5	
Tumor stage	T1	12	30	6	46.2	6	22.2	0.238
	T2	28	70	7	53.8	21	77.8	
Perineural invasion		25	62.5	7	53.8	18	66.7	0.663
Lymphovascular invasion		21	52.5	12	92.3	9	33.3	0.001*
Desmoplastic reaction	mature	15	37.5	0	0	15	55.6	< 0.001*
	intermediate	15	37.5	3	23.1	12	44.4	
	immature	10	25	10	76.9	0	0	
Depth of invasion (mm)	< 5	24	60	0	0	24	88.9	< 0.001*
	5+	16	40	13	100	3	11.1	
Lymphocytic infiltration	high	18	45	0	0	18	66.7	< 0.001*
	intermediate	13	32.5	4	30.8	9	33.3	
	low	9	22.5	9	69.2	0	0	

[1]χ^2 test;

*Fisher's exact probability test

Table 2. Risk factors for the occurrence of occult metastases – univariate logistic regression analysis

Characteristic		**Univariate logistic regression**				**Multivariate logistic regression**			
		OR	**95% CI**		**p**	**OR**	**95% CI**		**p**
Female sex		0.646	0.158	2.637	0.543	2.126	0.041	109.137	0.707
Age		1.007	0.945	1.072	0.832	1.100	.976	1.239	0.117
Alcohol		2.423	0.598	9.816	0.215	4.897	.082	290.940	0.446
Growth type – exophytic		2.327	0.600	9.028	0.222	0.495	0.036	6.881	0.601
Tumor grade	I	ref group			0.114				0.244
	II	9.000	0.969	83.583	0.053	31.774	.561	1798.965	0.093
	III	6.600	0.543	80.235	0.139	9.662	.241	386.897	0.228
Perineural invasion		0.583	0.151	2.256	0.435	.069	.004	1.266	0.072
Lymphovascular invasion		24.000	2.682	214.725	0.004	32.713	1.862	574.667	0.017
Constant						0.000			0.025

OR - odds ratio; 95% CI – 95% confidence interval;

Hosmer–Lemeshow test p = 0.135

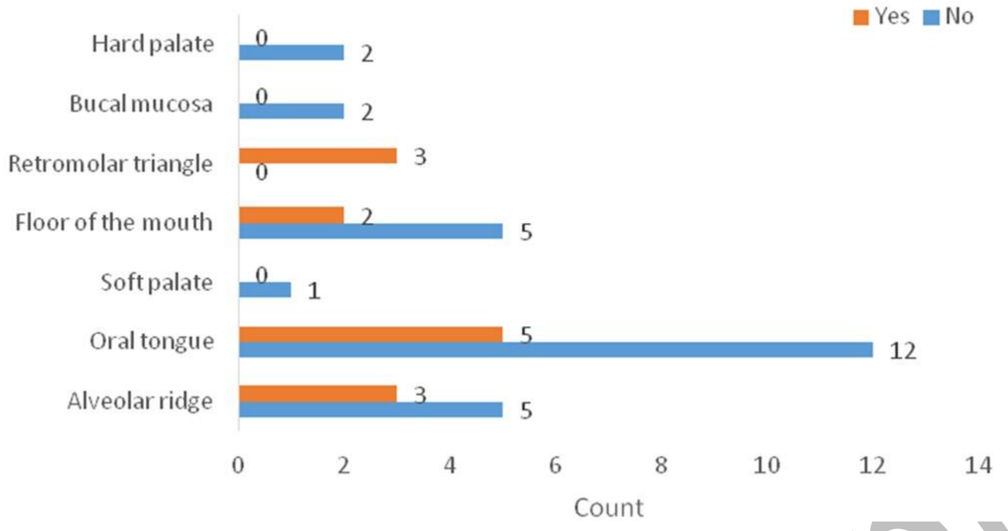

Figure 3. Tumor localization regarding the presence of occult metastases

