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ANALIZA LIMFOCITARNOG INFILTRATA KAO PREDIKTIVNOG FAKTORA POJAVE METASTAZA NA VRATU KOD ORALNOG SKVAMOCELULARNOG KARCINOMA

ANALYSIS OF LYMPHOCYTIC INFILTRATE AS A PREDICTIVE FACTOR OF NECK METASTASES IN ORAL SQUAMOUS CELL CARCINOMA

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Sažetak

Uvod: Karcinom pločasto slojevitog epitela usne duplje – oralni skvamocelularni karcinom (OSCC) – jeste invazivna patološka lezija epitela različitog stepena skvamozne diferencijacije, a odlikuje se ranim i ekstenzivnim metastazama, infiltracijom okolnih anatomskih struktura, pojavom učestalih recidiva i relativno niskom stopom petogodišnjeg preživljavanja, koja je manja od 50%.

Cilj ove studije je utvrđivanje korelacije između analize limfocitarnog infiltrata biopsijskih preparata sa patohistološki dokazanim metastazama oralnog skvamocelularnog karcinoma na postoperativnim preparatima disekata vrata.

Materijali i metode. Istraživanje je obuhvatilo 42 pacijenta koja su nakon patohistološke verifikacije oralnog skvamocelularnog karcinoma (OSCC) operisana na Odeljenju za maksilofacijalnu hirurgiju Klinike za dentalnu medicinu u Nišu u periodu od 2018. do 2022. godine. Histopatološka analiza podrazumevala je dubinu invazije tumora, histopatološku analizu limfnih nodusa disekata vrata, analizu limfocitarne infiltracije, prisustvo limfovaskularne i perineuralne invazije.

Rezultati. Utvrđeno je da se dubina invazije statistički značajno razlikuje u odnosu na stepen limfocitarne infiltracije ($p = 0,004$). Invazija preko 4 mm prisutna je kod 20,0% pacijenata sa visokom infiltracijom, kod 44,4% pacijenata sa srednjom infiltracijom i kod 85,7% pacijenata sa niskom infiltracijom.

Zaključak. Budući da rezultati studije ukazuju na to da analiza limfocitarnog infiltrata može biti pouzdan prediktivni faktor pojava metastaza u limfnim nodusima vrata, dalja istraživanja valja usmeriti ka povezivanju limfocitarne infiltracije i pojedinih biohemijskih i genetskih parametara na osnovu kojih se može stvoriti plan lečenja vrata N0 stadijuma oralnog skvamocelularnog karcinoma.

Gljučne reči: limfocitarna infiltracija, oralni skvamocelularni karcinom, hirurško lečenje

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Abstract

Introduction: Carcinoma of the stratified squamous epithelium of the oral cavity - oral squamous cell carcinoma (OSCC) is an invasive pathological lesion of the epithelium with varying degrees of squamous differentiation. It is characterized by early and extensive metastases, infiltration of adjacent anatomical structures, frequent recurrences, and a relatively low five-year survival rate of less than 50%.

The aim of the study to determine the correlation between the analysis of the lymphocytic infiltrate of biopsy specimens with pathohistologically proven metastases of oral squamous cellular carcinoma on postoperative neck dissection specimens.

Material and methods: The study included 42 patients with pathohistologically verified oral squamous cellular carcinoma who underwent surgery at the Department of Maxillofacial Surgery of the Clinic of Dental medicine in Niš in the period from 2018 to 2022. The histopathological analysis included the depth of tumor invasion, histopathological analysis of the lymph nodes of neck dissections, analysis of lymphocytic infiltration, and presence of lymphovascular and perineural invasion.

Results: It was determined that the depth of invasion is statistically significantly different regarding the degree of lymphocytic infiltration ($p=0,004$). Invasion of over 4mm was present in 20% of patients with high infiltration, 44.4% of patients with medium infiltration, and 85.7% of patients with low infiltration.

Conclusion: Given that the results of the study indicate that the analysis of the lymphocytic infiltrate can be a reliable predictive factor of the appearance of metastases in the lymph nodes of the neck, further research should be directed towards associating lymphocytic infiltration with certain biochemical and genetic parameters based on which a plan can be created for neck treatment in the N0 stage of oral squamous cellular carcinoma.

Key words: lymphocytic infiltration, oral squamous cell carcinoma, surgical treatment

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Uvod

Karcinom pločasto slojevitog epitela usne duplje – oralni skvamocelularni karcinom (OSCC) – jeste invazivna patološka lezija epitela različitog stepena skvamozne diferencijacije, a odlikuje se ranim i ekstenzivnim metastazama, infiltracijom okolnih anatomskih struktura, pojavom učestalih recidiva i relativno niskom stopom petogodišnjeg preživljavanja, koja je manja od 50%¹.

Statistički, OSCC predstavlja najučestaliji maligni tumor glave i vrata². OSCC je šesti karcinom po učestalosti kod muškaraca i deseti kod žena u razvijenim zemljama, dok je po učestalosti treći kod muškaraca, a četvrti kod žena u zemljama u razvoju³.

Hirurgija predstavlja apsolutno najefikasniji način lečenja OSCC-a. Radikalna ablacija tumora sa zdravom marginom od 4 mm do 10 mm uz limfadenektomiju vrata, adekvatnu rekonstrukciju nastalog defekta i postoperativnu onkološku terapiju predstavlja standard u lečenju OSCC-a⁴.

Veruje se da je prisustvom limfocita koji infiltriraju tumor (TILs) moguće predvideti odgovor na imunoterapiju, hemioterapiju, samu agresivnost tumora i predvideti pojavu metastaza na vratu^{5,6}. Naime, smatra se da prisustvo limfocita u samom tumoru, ali i njegovoj okolini, podržava imunski sistem pojedinca, naročito u aspektu antitumorskog odgovora.

Kvantifikacija limfocita uglavnom je relativno laka u tumorskom tkivu, ali kvalitativni aspekt infiltrata se razlikuje. U sastavu su prisutne ćelije i urođenog i adaptivnog imunskog odgovora i njihov odnos zavisi od tipa tumora, kao i od samog organa u kojem se odvija tumorski proces. Kumulativni podaci na osnovu pretkliničkih i kliničkih studija povezali su većinu leukocita sa dominantnim doprinosom u protumorskom ili antitumorskom odgovoru.

Na osnovu faktora u mišjim modelima identifikovani su leukociti mijeloidne loze, i to makrofagi, dendritske i supresorne ćelije. U tom kontekstu, antitumorske T-ćelije, koje migriraju u ovu sredinu, mogu biti aktivisane ili suprimirane. Imajući u vidu značaj unakrsnog ćelijskog odgovora u tumorskom tkivu, pod dejstvom T-limfocita makrofagi mogu biti polarizovani u pravcu M2 (protumorogeni fenotip) ili M1 (antitumorski fenotip)^{7,8}.

Introduction

Carcinoma of the stratified squamous epithelium of the oral cavity - oral squamous cell carcinoma (OSCC) is an invasive pathological lesion of the epithelium with varying degrees of squamous differentiation. It is characterized by early and extensive metastases, infiltration of adjacent anatomical structures, frequent recurrences, and a relatively low five-year survival rate of less than 50%¹.

Statistically, OSCC is the most common malignant tumor of the head and neck region². OSCC is the sixth most common carcinoma in men and the tenth most common in women in developed countries, i.e., the third and the fourth most common carcinoma in men and women, respectively, in developing countries³.

Surgery is undoubtedly the most effective way of treating OSCC. Radical tumor ablation with a healthy margin of 4–10 mm, along with lymphadenectomy of the neck, adequate reconstruction of the resulting defect, and postoperative oncological therapy is considered standard in the treatment of OSCC⁴.

It is believed that the presence of tumor-infiltrating lymphocytes (TILs) can predict the response to immuno- and/or chemotherapy, the aggressiveness of the tumor, as well as the appearance of metastases in the neck^{5,6}. Namely, it is considered that the presence of lymphocytes not only in the tumor but also in its surroundings supports the individual's immune system, especially in the aspect of anti-tumor response.

The quantification of lymphocytes in tumor tissue is generally rather easy. However, the qualitative aspect of the infiltrate differs. The composition includes cells of both the innate and adaptive immune response, and their ratio depends on the type of tumor, as well as on the organ itself in which the tumor process develops. Cumulative data from preclinical and clinical studies have associated most leukocytes with a dominant contribution in the pro- or antitumor response.

Based on their factors, the myeloid lineage leukocytes, namely macrophages, dendritic and suppressor cells, were identified in mouse models. In this context, anti-tumor T cells, which migrate into this environment, can be activated or suppressed. Considering the importance of the cross-cellular response in tumor tissue, macrophages can be polarized under the influence of T lymphocytes in the direction of M2 (pro-tumorigenic phenotype) or M1 (antitumor phenotype)^{7,8}.

Kliničke studije sugerišu adaptivni imunski odgovor posredovan T-limfocitima i B-limfocitima, koji obezbeđuje adekvatan antitumorski imunitet. Ekstenzivna infiltracija CD8+ T-limfocitima u visokom stepenu povezana je sa preživljavanjem i dobrim odgovorom na terapiju⁹⁻¹¹. Prisustvo CD4+ regulatornih T-limfocita (Treg) povezano je i sa dobrim i sa lošim ishodom¹²⁻¹⁴. Među ostalim subpopulacijama CD4+ T-limfocita, prisustvo Th1 ćelija (glavni ćelijski izvor interferona- γ) povezano je sa povoljnim kliničkim ishodom¹⁵, dok je prisustvo Th2 ćelija povezano sa smanjenim antitumorskim odgovorom¹⁶. Th 17 ćelije, odgovorne ćelije za proinflatornu citokinsku familiju interleukina¹⁷, imaju promenljive efekte u zavisnosti od ostalog citokinskog miljea. Ovo može biti objašnjeno mestom nastanka i samim tipom tumora¹⁸.

Cilj ove studije jeste da se utvrdi korelacija između analize limfocitarnog infiltrata biopsijskih preparata sa patohistološki dokazanim metastazama OSCC-a na postoperativnim preparatima disekata vrata.

Materijal i metode

Istraživanjem su obuhvaćena 42 pacijenta koja su nakon patohistološke verifikacije OSCC-a operisana na Odeljenju za maksilofacijalnu hirurgiju Klinike za dentalnu medicinu u Nišu u periodu od 2018. do 2022. godine.

Iz studije su isključeni pacijenti sa recidivantim tumorskim procesom, pacijenti sa patohistološki potvrđenim nalazom OSCC-a i prisutnim udaljenim sekundarnim deponitima, pacijenti kojima je intervencija u opštoj endotrahealnoj anesteziji (OETA) bila kontraindikovana zbog komorbiditeta, kao i pacijenti koji su zbog inicijalno inoperabilnog tumorskog procesa bili primarno onkološki tretirani i prevedeni u operabilno stanje.

Analizirani su anamnestički podaci (porodična i lična anamneza), period od prvih subjektivnih tegoba do operativnog zahvata i loše navike. Valja napomenuti da je porodična anamneza zabeležena kao pozitivna isključivo u prvoj liniji srodstva.

Analiza kliničkog nalaza podrazumevala je preciznu anatomsku lokaciju tumora, veličinu tumora, prisustvo uvećanih limfnih nodusa vrata, kao i TNM klasifikaciju.

Histopatološka analiza podrazumevala je dubinu invazije tumora (DOI), histopatološku analizu limfnih nodusa disekata vrata, analizu limfocitarne infiltracije i prisustvo limfovaskularne (LVI) i perineuralne invazije (PNI).

Clinical studies suggest an adaptive immune response mediated by T and B lymphocytes, which provides adequate antitumor immunity. Extensive infiltration of CD8+ T lymphocytes is highly associated with survival and good response to therapy⁹⁻¹¹. The presence of CD4+ regulatory T lymphocytes (Treg) is linked with both good and poor outcome¹²⁻¹⁴. Among other CD4+ T lymphocyte subpopulations, the presence of Th1 cells (the main cellular source of interferon- γ) is associated with a favourable clinical outcome¹⁵, whereas the presence of Th2 cells is associated with a reduced antitumor response¹⁶. Th17 cells, i.e., cells responsible for the pro-inflammatory cytokine family of interleukins¹⁷, have variable effects depending on the rest of the cytokine milieu. This can be explained by the place of origin and type of the tumor¹⁸.

The aim of the study was to determine the correlation between the analysis of the lymphocytic infiltrate of biopsy specimens with pathohistologically proven metastases of OSCC on postoperative neck dissection specimens.

Material and methods

The study included 42 patients with pathohistologically verified OSCC who had undergone surgery at the Department of Maxillofacial Surgery of the Dental Medicine Clinic in Niš in the period from 2018 to 2022.

The study excluded patients with a recurrent tumor process, patients with pathohistologically confirmed OSCC and remote secondary deposits, patients in whom surgery under general endotracheal anaesthesia (GETA) had been contraindicated due to comorbidities, as well as patients who, due to an initially inoperable tumor process, received oncological treatment that brought them into operable condition.

The analysis included anamnestic data (family and personal medical history), the period between first symptoms and surgery, as well as bad habits. It should be noted that the family history was recorded as positive in the first line of kinship exclusively.

The analysis of clinical findings included the exact anatomical location of the tumor, the size of the tumor, the presence of enlarged neck lymph nodes, and the TNM classification.

The histopathological analysis included the depth of tumor invasion (DOI), histopathological analysis of the lymph nodes of neck dissections, analysis of lymphocytic infiltration, and presence of lymphovascular (LVI) and perineural invasion (PNI).

Statistička obrada podataka

Podaci su prikazani u vidu apsolutnih i relativnih brojeva. Poređenje kategorijskih obeležja između grupa vršeno je Hi kvadrat testom i Fišerovim testom egzaktno verovatnoće. Hipoteza je testirana, a prag značajnosti postavljen je na $p < 0,05$. Statistička obrada podataka vršena je u programskom paketu EPI INFO v 7.2.2.6.

Rezultati

U Tabeli 1 prikazane su demografske i kliničke karakteristike ispitivane populacije, te su predstavljene u odnosu na limfocitarnu infiltraciju.

Većina pacijenata bila je starosti od 50 do 60 godina (45,2%) i muškog pola (59,5%). Polovina pacijenata (52,4%) imala je dubinu invazije > 4 mm. Perineuralne invazije bile su prisutne kod 40,5% pacijenata. Limfovaskularne invazije bile su prisutne kod 52,4% pacijenata. Distribucija lokalizacije promena je sledeća: jezik kod 40,5% pacijenata, jezik i pod usta kod 19,0% pacijenata, sluzokoža obraza kod 11,9% pacijenata, pod usta kod 11,9% pacijenata, alveolarni nastavak kod 9,5% pacijenata, tvrdo nepce kod 7,1% pacijenata. Prisutni su sledeći stadijumi bolesti u ispitivanoj populaciji: stadijum I kod 28,6% pacijenata, stadijum II kod 28,6% pacijenata, stadijum III kod 23,8% pacijenata i stadijum IV kod 19,0% pacijenata.

Utvrđeno je da se starost i pol ne razlikuju statistički značajno u odnosu na stepen limfocitarne infiltracije ($p = 0,991$, odnosno $p = 0,303$). Dubina invazije statistički se značajno razlikuje u odnosu stepena limfocitarne infiltracije ($p = 0,004$) (Grafikon 1). Invazija preko 4 mm prisutna je kod 20,0% pacijenata sa visokom infiltracijom, 44,4% pacijenata sa srednjom infiltracijom i 85,7% pacijenata sa niskom infiltracijom. Učestalost perineuralne invazije, lokalizacija i stadijum bolesti ne razlikuju se statistički značajno u odnosu na stepen limfocitarne infiltracije ($p = 0,882$, $p = 0,390$, odnosno $p = 0,085$).

U ispitivanoj populaciji metastaze su prisutne kod 27 pacijenata (64,3%). Utvrđeno je da starost i pol nisu statistički značajno povezani sa prisustvom metastaza ($p = 0,466$, odnosno $p = 0,923$) (Tabela 2). Dubina invazije statistički je značajno povezana sa prisustvom metastaza ($p = 0,045$). Metastaze su prisutne kod 70,4% pacijenata sa dubinom invazije > 4 mm.

Statistical data processing

Data are presented in the form of absolute and relative numbers. The comparison of categorical characteristics between groups was performed using the Chi-square test and Fisher's exact probability test. The hypothesis was tested with a significance threshold of $p < 0.05$. Statistical data processing was performed in EPI INFO v 7.2.2.6 software pack.

Results

Table 1 shows the demographic and clinical characteristics of the studied population in relation to lymphocytic infiltration.

Most patients were 50–60 years old (45.2%) and of male sex (59.5%). Half of the patients (52.4%) had a depth of invasion > 4 mm. Perineural invasions were present in 40.5% of the patients. Lymphovascular invasions were present in 52.4% of the patients. The distribution of the localization of changes was the following: tongue (40.5%), tongue and floor of the oral cavity (19.0%), buccal mucosa (11.9%), floor of the oral cavity (11.9%), alveolar process (9.5%), and hard palate (7.1%). The following disease stages were present in the studied population: stage I (28.6%), stage II (28.6%), stage III (23.8%), and stage IV (19.0%).

The study revealed that age and gender did not differ statistically significantly regarding the degree of lymphocytic infiltration ($p=0.991$ and $p=0.303$, respectively). The depth of invasion was statistically significantly different regarding the degree of lymphocytic infiltration ($p=0.004$) (Graph1). Invasion of over 4mm was present in 20% of patients with high infiltration, 44.4% of patients with medium infiltration, and 85.7% of patients with low infiltration. The frequency of perineural invasion, localization and stage of the disease did not differ statistically significantly regarding the degree of lymphocytic infiltration ($p=0.882$, $p=0.390$, $p=0.085$, respectively).

In the studied population, metastases were present in 27 patients (64.3%). It was found that age and gender were not statistically significantly associated with the presence of metastases ($p=0.466$ and $p=0.923$, respectively) (Table 2), unlike the depth of invasion ($p=0.045$). Metastases were present in 70.4% of patients with the depth of invasion > 4 mm.

Perineuralane invazije, limfovaskularne invazije i lokalizacija ne razlikuju se statistički značajno u odnosu na prisustvo metastaza ($p=1,000$, $p=0,263$, odnosno $p=0,980$). Stadijum bolesti statistički se značajno razlikuje u odnosu na prisustvo metastaza ($p < 0,001$). Metastaze su prisutne kod 11,1% pacijenata sa stadijumom 1; 18,5% pacijenata sa stadijumom 2; 25,9% pacijenata sa stadijumom 3 i 44,4% pacijenata sa stadijumom 4.

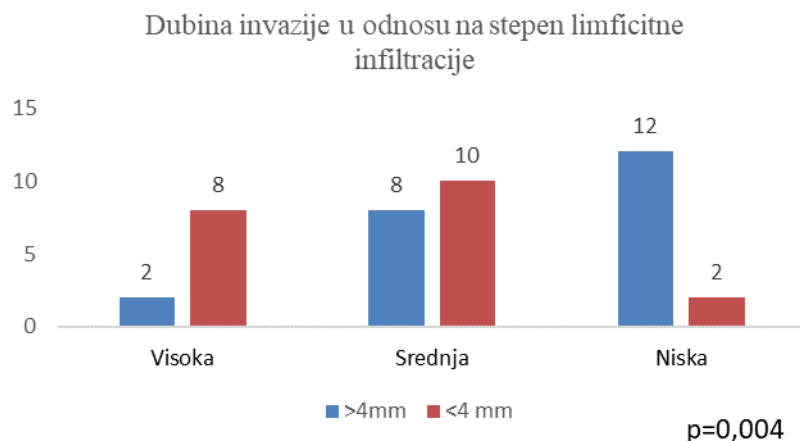
Perineural invasions, lymphovascular invasions, and localization did not differ statistically significantly regarding the presence of metastases ($p=1.000$, $p=0.263$, $p=0.980$, respectively). The stage of the disease was statistically significantly different in relation to the presence of metastases ($p<0.001$). Metastases were present in 11.1% of patients with stage I, 18.5% of patients with stage II, 25.9% of patients with stage III, and 44.4% of patients with stage IV.

Tabela 1. Kliničke i demografske karakteristike u odnosu na stepen limfocitne infiltracije
Table 1. Clinical and demographic characteristics in relation to the degree of lymphocytic infiltration

Karakteristika / Characteristic	Ukupno / Total		Limfocitarna infiltracija / Lymphocytic infiltration						p ¹
			Visoka High		Srednja Medium		Niska Low		
	n	%	n	%	n	%	n	%	
Starost Age									
< 50	16	38.1	4	40.0	7	38.9	5	35.7	0.991
50-60	19	45.2	4	40.0	8	44.4	7	50.0	
> 70	7	16.7	2	20.0	3	16.7	2	14.3	
Pol Sex									
Muški Male	25	59.5	8	80.0	10	55.6	7	50.0	0.303
Ženski Female	17	40.5	2	20.0	8	44.4	7	50.0	
Dubina invazije Depth of invasion									
> 4mm	22	52.4	2	20.0	8	44.4	12	85.7	0.004
< 4 mm	20	47.6	8	80.0	10	55.6	2	14.3	
Perineuralaneinvazije Perineural invasion									
Da / Yes	17	40.5	4	40.0	8	44.4	5	35.7	0.882
Ne / No	25	59.5	6	60.0	10	55.6	9	64.3	
Limfovaskularneinvazije Lymphovascular invasion									
Da / Yes	22	52.4	2	20.0	10	55.6	10	71.4	0.042
Ne / No	20	47.6	8	80.0	8	44.4	4	28.6	
Lokalizacija Localization									
Jezik Tongue	17	40.5	4	40.0	8	44.4	5	35.7	0.390 ^a
Jezik i pod usta Tongue and floor of oral cavity	8	19.0	2	20.0	3	16.7	3	21.4	
Sluzokoža obraza Buccal mucosa	5	11.9	0	0.0	4	22.2	1	7.1	
Pod usta Floor of oral cavity	5	11.9	1	10.0	3	16.7	1	7.1	
Alveolarni nastavak Alveolar process	4	9.5	1	10.0	0	0.0	3	21.4	
Tvrdo nepce Hard palate	3	7.1	2	20.0	0	0.0	1	7.1	
Stadijum Stage									
I	12	28.6	6	60.0	4	22.2	2	14.3	0.085 ^a
II	12	28.6	2	20.0	7	38.9	3	21.4	
III	10	23.8	2	20.0	5	27.8	3	21.4	
IV	8	19.0	0	0.0	2	11.1	6	42.9	

¹ Hi-kvadrat test, a Fišherov test egzaktne verovatnoće

¹ Chi-square test, a Fisher's exact probability test



Grafikon 1. Dubina invazije u odnosu na stepen limfocitne infiltracije
Graph 1. The depth of invasion regarding the degree of lymphocytic infiltration

Tabela 2. Kliničke i demografske karakteristike u odnosu na prisustvo metastaza
Table 2. Clinical and demographic characteristics regarding the presence of metastases

Karakteristika / Characteristic	Prisustvo metastaza / Metastases				p ¹
	Prisutne / Existent		Odsutne / Non-existent		
	n	%	n	%	
Starost Age					
< 50	8	29.6	7	46.7	0.466
50-60	14	51.9	5	33.3	
> 70	5	18.5	3	20.0	
Pol Sex					
Muški Male	18	66.7	9	60.0	0.923
Ženski Female	9	33.3	6	40.0	
Dubina invazije Depth of invasion					
>4mm	19	70.4	5	33.3	0.045
<4 mm	8	29.6	10	66.7	
Perineuralneinvazije Perineural invasion					
Da Yes	12	44.4	6	40.0	1.000
Ne No	15	55.6	9	60.0	
Limfovaskularneinvazije Lymphovascular invasion					
Da Yes	19	70.4	7	46.7	0.263
Ne No	8	29.6	8	53.3	
Lokalizacija Localisation					
Jezik Tongue	10	37.0	5	33.3	0.980 ^a
Jezik i pod usta Tongue and floor of oral cavity	5	18.5	3	20.0	
Sluzokoža obraza Buccal mucosa	4	14.8	2	13.3	
Pod usta Floor of oral cavity	5	18.5	2	13.3	
Alveolarni nastavak Alveolar process	2	7.4	2	13.3	
Tvrdo nepce Hard palate	1	3.7	1	6.7	
Stadijum Stage					
I	3	11.1	8	53.3	<0.001 ^a
II	5	18.5	6	40.0	
III	7	25.9	1	6.7	
IV	12	44.4	0	0.0	

¹ Hi-kvadrat test, a Fišerov test egzaktne verovatnoće

¹ Chi-square test, a Fisher's exact probability test

Diskusija

Limfogeno diseminacija u regionalne limfne noduse vrata predstavlja glavni način metastaziranja OSCC-a, a takođe i bitan faktor u definitivnoj prognozi bolesti. Broj i veličina uvećanih metastatskih limfonodusa, prisustvo ekstrakapsularne ekstenzije i angažovanje kaudalnih regija vrata direktno utiču na prognozu bolesti¹⁹. Uvidom u dostupnu literaturu, utvrđeno je da 50% pacijenata sa OPK-om ima patohistološki potvrđene metastaze u regionalnim limfnim nodusima vrata. Takođe, potvrđeno je da u 5,3% slučajeva prisustvo uvećanih limfonodusa na vratu predstavlja prvi znak prisustva OSCC-a u usnoj duplji²⁰. Definitivna prognoza pacijenata sa OSCC-om i metastazama u regionalnim limfnim nodusima vrata znatno je gora, dok je verovatnoća pojave udaljenih metastaza kod pomenute grupe daleko veća²¹.

DOI predstavlja jedan od važnijih prognostičkih parametara pojave metastaza na vratu kod OSCC-a. U mnogobrojnim analizama DOI predstavlja nezavisni prognostički faktor koji je povezan sa veličinom tumora, naročito kod pacijenata sa T1–T2 stadijumom²². Istraživanje koje je obuhvatilo 161 pacijenta sa OPK-om i kliničkim N0 nalazom na vratu ukazalo je na postojanje korelacije između DOI i pojave metastaza u regionalnim limfnim nodusima vrata²³. Antonio lo Casto i sar.²⁴ prikazuju rezultate kojima se dokazuje da je DOI najpouzdaniji prognostički parametar OPK-a u stadijumu T1–T2. Granična vrednost DOI u literaturi varira od 1,5 mm do 10 mm u različitim studijama, tako da je za signifikantnu srednju vrednost preuzeta vrednost od 4 mm, koja predstavlja apsolutnu indikaciju za sprovođenje selektivnih disekcija vrata²⁵. Rezultati naše studije pokazuju korelaciju između DOI i prisustva metastaza u limfnim nodusima disekata vrata.

LVI i PNI su po mnogobrojnim autorima, takođe, bitan prognostički faktor povezan sa kliničkim karakteristikama OSCC-a. Teorijski, pojam vaskularne invazije podrazumeva da je određeni broj tumorskih ćelija prodro u lumen krvnog suda i predstavlja prvi korak u metastaziranju. PNI može biti izolovana ili u sklopu LVI. Wu i sar.²⁶ su grupu od 171 pacijenta sa planocelularnim karcinomom jezika stadijuma T1–T2 podelili u dve grupe u zavisnosti od prisustva ili odsustva LVI i PNI. Uočena je statistički značajna pojava metastaza na vratu i veća pojava recidiva u grupi pacijenata kod kojih su LVI i PVI bili pozitivni.

Discussion

Lymphatic dissemination in the regional lymph nodes of the neck is the main way of OSCC metastasizing and an important factor in the definite prognosis of the disease. The number and size of enlarged metastatic lymph nodes, the presence of extracapsular extension, and the involvement of caudal regions of the neck directly affect the prognosis of the disease¹⁹. Data from the available literature reveal that 50% of patients with OSCC have pathohistologically confirmed metastases in the regional lymph nodes of the neck. Based on the findings in 5.3% of cases, it was also confirmed that the presence of enlarged lymph nodes in the neck region is the first sign of OSCC in the oral cavity²⁰. Patients with OSCC have a poor prognosis of the appearance of metastases in the regional lymph nodes of the neck, whereas the probability of the occurrence of distant metastases in these patients is much higher²¹.

DOI represents one of the most important prognostic parameters for the appearance of neck metastases in OSCC. In numerous analyses, DOI is an independent prognostic factor that is associated with tumor size, especially in patients with T1–T2 stage²². A study that included 161 patients with OSCC and clinical N0 findings in the neck indicated a correlation between DOI and metastases in the regional lymph nodes of the neck²³. Antonio lo Casto et al.²⁴ presented results proving that DOI is the most reliable prognostic parameter of OSCC in stages T1–T2. In literature, DOI values vary from 1.5 to 10 mm in different studies. Thus, a mean value of 4 mm was taken as significant, and it represents an absolute indication for performing selective neck dissections²⁵. The results of our study show a correlation between DOI and the presence of metastases in the lymph nodes of neck dissection specimens.

LVI and PNI are also considered important prognostic factors associated with the clinical characteristics of OSCC. Theoretically, the concept of vascular invasion implies that a certain number of tumor cells have penetrated the lumen of a blood vessel, which represents the first step in metastasizing. PNI can be isolated or part of LVI. Wu et al.²⁶ divided a group of 171 patients with T1–T2 squamous cell carcinoma of the tongue into two groups depending on the presence or absence of LVI and PNI. A statistically significant occurrence of metastases in the neck and a higher incidence of recurrence was observed in the group of patients in whom LVI and PVI were positive.

Yeh i sar.²⁷ u studiji, koja je obuhvatila 253 pacijenta sa OSCC-om u ranom stadijumu, konstatuju postojanje većeg procenta pojave recidivantnih metastatskih promena na vratu kod pacijenata kod kojih su LVI i PNI bili pozitivni. Rezultati naše studije ne ukazuju na postojanje statističke značajnosti između LVI i PVI i pojave metastaza u limfnim nodusima vrata. Ovakav rezultat pravdamo malim brojem pacijenata sa pozitivnim LVI i PVI koji su uključeni u studiju, budući da je velik broj pacijenata sa OPK-om bio isključen iz studije.

Kvantifikacija, kvalifikacija i lokacija TILS-a predstavljaju signifikantne prognostičke faktore kod pacijenata sa karcinomom dojke, digestivnog trakta, limfoma i želuca, ali su takođe u koleraciji sa odgovorom na onkološku terapiju i prognozom. Kod OPK-a, tumorska infiltracija antigenprezentujućim ćelijama, npr. makrofagima, Langerhansovim ćelijama i CD1 pozitivnim dendritičnim ćelijama, povezana je sa ponašanjem tumora i preživljavanjem pacijenata. Pojava CD4 limfocita u infiltratu predstavlja povoljan prognostički znak, za razliku od infiltracije makrofagima, koja je povezana sa pojavom metastaza u regionalnim limfnim nodusima vrata²⁸. Pojava CD68 pozitivnih makrofaga predstavlja loš prognostički znak i povezuje se sa pojavom metastaza u regionalnim limfnim nodusima vrata²⁹. Rezultati naše studije pokazuju korelaciju gustine limfocitarnog infiltrata i pojave metastaza u limfnim nodusima vrata, kao i korelaciju pomenutog parametra sa DOI.

Zaključak

U našoj studiji su prikazani preliminarni rezultati, koji ukazuju na povezanost limfocita koji infiltriraju tumor i kliničkih karakteristika OSCC-a. Rezultati studije ukazuju na to da analiza limfocitarnog infiltrata može biti pouzdan prediktivni faktor pojava metastaza u limfnim nodusima vrata. Dalja istraživanja valja usmeriti ka povezivanju limfocitarne infiltracije i pojedinih biohemijskih parametara i genetskih promena, na osnovu kojih se može stvoriti plan lečenja vrata N0 stadijuma OPK-a, za koji još uvek u hirurgiji glave i vrata ne postoji jasno decidirani stav.

Zahvalnica: Nema

Sukob interesa: Nema

In a study that included 253 patients with OSCC in the early stage, Yeh et al.²⁷ suggested a higher percentage of recurrent metastatic changes in the neck in patients in whom LVI and PNI were positive. The results of our study do not indicate the existence of statistical significance between LVI and PVI and the occurrence of metastases in the lymph nodes of the neck. This result is justified by the small number of patients with positive LVI and PVI included in the study, given that many patients with OSCC were excluded from the study.

The quantification, qualification, and location of TILs is a significant prognostic factor in patients with carcinoma of the breast, digestive tract, and lymphoma, but it is also in correlation with the response to oncological therapy and prognosis. In OSCC, tumor infiltration by antigen-presenting cells such as macrophages, Langerhans cells, and CD1-positive dendritic cells is associated with tumor behaviour and patient survival. The appearance of CD4 lymphocytes in the infiltrate is a favourable prognostic sign, unlike infiltration by macrophages which is associated with the appearance of metastases in the regional lymph nodes of the neck²⁸. The appearance of CD68-positive macrophages is a bad prognostic sign and is associated with metastases in the regional lymph nodes of the neck²⁹. The results of our study show a correlation between the density of the lymphocytic infiltrate and the occurrence of metastases in the lymph nodes of the neck, as well as a correlation of the mentioned parameter with DOI.

Conclusion

The preliminary results of our study indicate a relationship between tumor-infiltrating lymphocytes and the clinical characteristics of OSCC. The results of the study suggest that the analysis of the lymphocytic infiltrate can be a reliable predictive factor for the appearance of metastases in the lymph nodes of the neck. Further research should be directed towards associating lymphocytic infiltration with certain biochemical parameters and genetic changes based on which a plan can be created for neck treatment in the N0 stage of OSCC, for which there is still no clearly defined viewpoint in head and neck surgery.

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LITERATURA /REFERENCES

- Norouzi A, Liaghat M, Bakhtiyari M, Varnosfaderani SMN, Zalpoor H, Nabi-Afjadi M, Molania T. The potential role of COVID-19 in progression, chemo-resistance, and tumor recurrence of oral squamous cell carcinoma (OSCC). *Oral Oncology* 2023; 106483.
- Badwelan M, Muaddi H, Ahmed A, Lee T.K, Simon D.T. Oral Squamous Cell Carcinoma and Concomitant Primary Tumors, What Do We Know? A Review of the Literature. *Current Oncology* 2023; 3721-3734.
- Katelyn OS, Mazul Z.A., Puram VS. Changing epidemiology of oral cavity cancer in the United States. *Otolaryngology–Head and Neck Surgery* 2023; 761-768.
- Zittel S, Moratin J, Horn D, Metzger K, Ristow O, Engel M, Mrosek J, Freier K, Hoffmann J, Freudlsperger C. Clinical outcome and prognostic factors in recurrent oral squamous cell carcinoma after primary surgical treatment: a retrospective study. *Clinical Oral Investigations* 2022; 1-10.
- El Bairi K, Haynes HR, Blackley E, Fineberg S, Shear J, Turner S. The tale of TILs in breast cancer: A report from The International Immunology Biomarker Working Group. *NPJ Breast Cancer*. 2021;7(1):150.
- Baxevanis CN, Fortis SP, Perez SA. The balance between breast cancer and the immune system: challenges for prognosis and clinical benefit from immunotherapies. *Semin. Cancer Bio.* <https://doi.org/10.1016/j.semcancer.2019.12.018>
- Galon J, Angell HK, Bedognetti D. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. *Immunity*. 2013; 39:11–26.
- Coussens LM, Pollard JW. Leukocytes in mammary development and cancer. *Cold Spring Harb Perspect Biol.* 2011; 3: pii: a003285.
- DeNardo DG, Barreto JB, Andreu P. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell*. 2009; 16:91–102.
- Mahmoud SM, Paish EC, Powe DG et al. Tumour-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. *J Clin Oncol.* 2011; 29:1949–1955.
- Liu S, Foulkes WD, Leung S. Prognostic significance of FOXP3+ tumor infiltrating lymphocytes in breast cancer depends on estrogen receptor and human epidermal growth factor receptor-2 expression status and concurrent cytotoxic T-cell infiltration. *Breast Cancer Res.* 2014; 16:432.
- Seo AN, Lee HJ, Kim EJ. Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. *Br J Cancer.* 2013; 109:2705–2713.
- Bates GJ, Fox SB, Han C. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J Clin Oncol.* 2006; 24:5373–5380.
- Gobert M, Treilleux I, Bendriss-Vermare N et al. Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. *Cancer Res.* 2009; 69:2000–2009.
- West NR, Kost SE, Martin SD. Tumour-infiltrating FOXP3(+) lymphocytes are associated with cytotoxic immune responses and good clinical outcome in oestrogen receptor-negative breast cancer. *Br J Cancer.* 2013; 108:155–162.
- Gu-Trantien C, Loi S, Garaud S. CD4(+) follicular helper T cell infiltration predicts breast cancer survival. *J Clin Invest.* 2013; 123:2873–2892.
- Teschendorff AE, Gomez S, Arenas A et al. Improved prognostic classification of breast cancer defined by antagonistic activation patterns of immune response pathway modules. *BMC Cancer.* 2010; 10:604.
- Qi W, Huang X, Wang J. Correlation between Th17 cells and tumor microenvironment. *Cell Immunol* 2013; 285:18–22.
- Bobdey S, Sathwara J, Jain A, Saoba S, Balasubramaniam G. Squamous cell carcinoma of buccal mucosa: An analysis of prognostic factors. *South Asian J Cancer.* 2018;7:49–54.
- Forghani R, Yu E, Levental M, Som PM, Curtin HD. Imaging evaluation of lymphadenopathy and patterns of lymph node spread in head and neck cancer. *Expert Rev Anticancer Ther.* 2015;15:207–24.
- Akhter M, Hossain S, Rahman QB, Molla MR. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis. *Journal of oral and maxillofacial pathology: JOMFP.* 2011;15:168–76.
- Marzouki HZ, Bukhari AF, Al-Ghamdi DA, Abdullah RM, Al-Hajeili M, Khayyat S, Alzahrani RM, Alotaibi YR, Al-Wassia R, Al-Marzouki H, Merdad M. Worst pattern of invasion and other histopathological features in oral cancer as determinants of prognosis and survival rate: A retrospective cohort analysis. *Oncol Lett.* 2023 Jan 4;25(2):75
- Li Y, Liu K, Ke Y, Zeng Y, Chen M, Li W, Liu W, Hua X, Li Z, Zhong Y, Xie C, Yu H. Risk Factors Analysis of Pathologically Confirmed Cervical Lymph Nodes Metastasis in Oral Squamous Cell Carcinoma Patients with Clinically Negative Cervical Lymph Node: Results from a Cancer Center of Central China. *J Cancer.* 2019 Jun 2;10(13):3062-3069.
- Lo Casto A, Cannella R, Taravella R, Cordova A, Matta D, Campisi G, Attanasio M, Rinaldi G, Rodolico V. Diagnostic and prognostic value of magnetic resonance imaging in the detection of tumor depth of invasion and bone invasion in patients with oral cavity cancer. *Radiol Med.* 2022 Dec;127(12):1364-1372.
- Riemenschnitter CE, Morand GB, Schouten CS, Rupp NJ, Balermipas P, Gander T, Broglie Däppen MA. Need for adjuvant radiotherapy in oral cancer: depth of invasion rather than tumor diameter. *Eur Arch Otorhinolaryngol.* 2023 Jan;280(1):339-346.
- Wu K, Yang X, Li L, Ruan M, Liu W, Lu W. et al. Neurovascular Invasion and Histological Grade Serve as the Risk Factors of Cervical Lymph Node Metastases in Early Tongue Squamous Cell Carcinoma. *Mol Neurobiol.* 2016;53:2920–6.
- Yeh CF, Li WY, Yang MH, Chu PY, Lu YT, Wang YF, Chang PM, Tai SK. Neck observation is appropriate in T1-2, cN0 oral squamous cell

- carcinoma without perineural invasion or lymphovascular invasion. *Oral Oncol.* 2014 Sep;50(9):857-62
28. Wolf GT, Chepeha DB, Bellile E, Nguyen A, Thomas D, McHugh J; University of Michigan Head and Neck SPORE Program. Tumor infiltrating lymphocytes (TIL) and prognosis in oral cavity squamous carcinoma: a preliminary study. *Oral Oncol.* 2015 Jan;51(1):90-5
29. Xue Y, Song X, Fan S, Deng R. The role of tumor-associated macrophages in oral squamous cell carcinoma. *Front Physiol.* 2022 Aug 29;13:959747