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Assessment of Density of Neovascularization in Lower Lip Squamous Cell Carcinoma in Relation To Neoplasm Differentiation Grade in Patients with and without Neck Lymph Nodes Metastasis

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Abstract

BACKGROUND: Squamous cell carcinoma (SCC) of the lower lip is a relatively rare carcinoma, with the incidence of 1 to 2%, but it is the most common carcinoma in the oral cavity accounting for 25-30% of all malignant oral tumours.

AIM: This study aimed to determine the role of neovascularisation in the process of tumour progression.

METHODS: We analysed the surgical specimens obtained from 60 patients with squamous cell carcinoma (SCC) of the lower lip. The examined group consisted of 45 specimens of patients without metastasis and 15 specimens of patients with metastasis in the regional lymph nodes. Histopathological slides were immunohistochemically stained with an antibody against CD34 and by hematoxylin & eosin staining for routine histopathological examination.

RESULTS: The results obtained showed a statistically significant difference in the density of neovascularisation between groups of the SCC with different grade of differentiation (Kruskal-Wallis test: H (2, N = 60) = 30.0943, p = 0.00001). Statistical analysis also showed a significant difference in the density of vascularisation of lower lip SCC between patients without metastasis and patients with neck metastasis (Mann-Whitney U test, p = 0.000198). Applying Pearson's chi-square test, we found a highly significant statistical difference in grade of SCC differentiation in patients with and without neck metastasis (p = 0.0000).

CONCLUSION: In conclusion, the density of neoangiogenesis is increased in tumours with poorer differentiation and in patients with neck metastasis. So, the density of neovascularisation of the primary lip SCC may predict the tumour progression.

Introduction

Squamous cell carcinoma (SCC) of the lower lip is a relatively rare carcinoma, which incidence ranges between 1% and 2%, but it is the most common carcinoma in the oral cavity accounting for approximately 25-30% of all malignant oral tumours. Over the last years, an increasing tendency of this carcinoma has been observed [1], [2], [3].

Metastases in the neck lymph nodes from SCC of the lower lip are found in less than 20% of patients, and they are more frequent in patients with worse neoplasm differentiation. In patients with smaller tumours (pT1 and pT2) at the moment of diagnosis, metastases are present in about 8% [4], [5], [6], [7].

The density of the newly formed blood vessels from the preexisting capillaries (neoangiogenesis), has been examined in squamous cell carcinoma in different locations of the body by many authors. Neoangiogenesis as a parameter can be a good indicator of tumour progression or its aggressiveness, and consequently of disease prognosis [8], [9], [10], [11].

This study aimed to determine the density of neovascularization at the invasive front in lower lip

squamous cell carcinomas in patients with and without neck metastasis about the grade of neoplasm histological differentiation. We also aimed to determine the role of neovascularization in the process of tumour progression, that is, in the onset of neck metastasis.

Material and Methods

The study included surgical specimens obtained from 60 patients with squamous cell carcinoma of the lower lip, who underwent surgical treatment at the University Clinic of Maxillofacial Surgery in Skopje. This was a retrospective study, for which purposes the archival material from the Institute of Pathology was retrieved, paraffin blocks, histopathological slides and histopathological reports were used. Specimens were histopathologically analysed at the Institute of Pathology, Medical Faculty in Skopje.

The examined group consisted of 60 specimens of patients with squamous cell carcinoma of the lower lip, of which 45 specimens of patients without regional lymph nodes metastasis and 15 specimens of patients with regional lymph nodes metastasis (Figure 1 and 2).

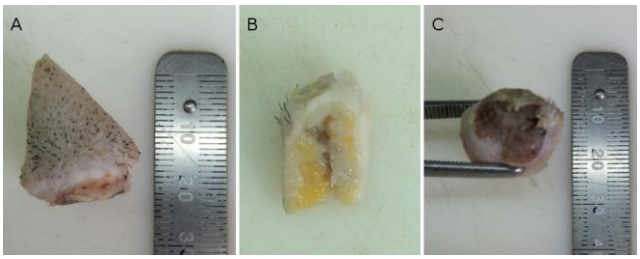

Figure 1: a) Lower lip squamous cell carcinoma. Triangle-shaped excision with central ulceration and crust over it; b) Side view of the specimen; c) another specimen of SCC with central ulceration

The density of the blood vessels (neoangiogenesis) in the stroma of the invasive front of the squamous cell carcinoma was determined on histological sections from both, the examined and the control group of specimens. From the resection margins of every triangle surgical excisions (60 cases) with low lip carcinoma, a specimen of non-tumour tissue (normal healthy tissue) was taken for the control group.

Immunohistochemical staining was performed with the standard procedure used at the Institute of Pathology, Medical Faculty in Skopje, by using Immunoperoxidase LSAB + system and specific primary monoclonal antibodies against CD 34 DAKO, Monoclonal Mouse Anti-Human CD 34 Classe II Clon QBEnd 10, code M716501-2.

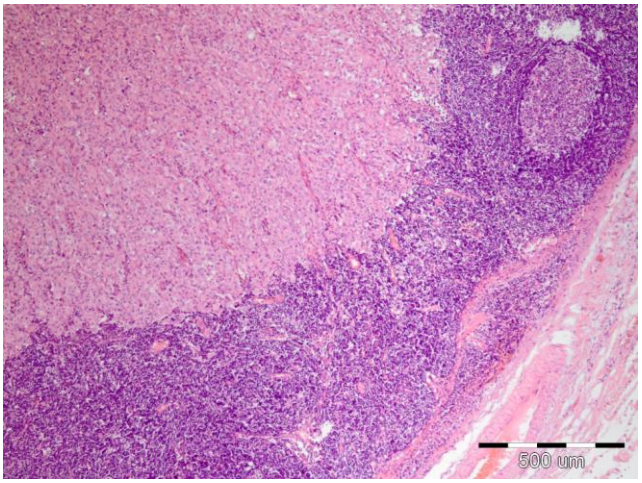

Figure 2: Neck lymph node with a metastatic deposit from a patient with moderately differentiated lower lip squamous cell carcinoma (HE 10 x 10)

Negative controls were carcinoma tissues in which primary antibody was omitted, and positive controls were considered carcinoma tissues with high expression of relevant proteins.

Staining with CD34 antibody was used for visualisation of tumour neoangiogenesis in the stroma at the invasive neoplasm front by endothelial brown staining cells in the blood vessels. At a magnification of 10 x 4, areas with the highest vascular density (hot spots) were identified, and at a magnification of 10 x 40, blood vessels were counted including groups of proliferative endothelial cells with clearly formed lumen in a set of 10 visual fields (Figure 3 and 4).

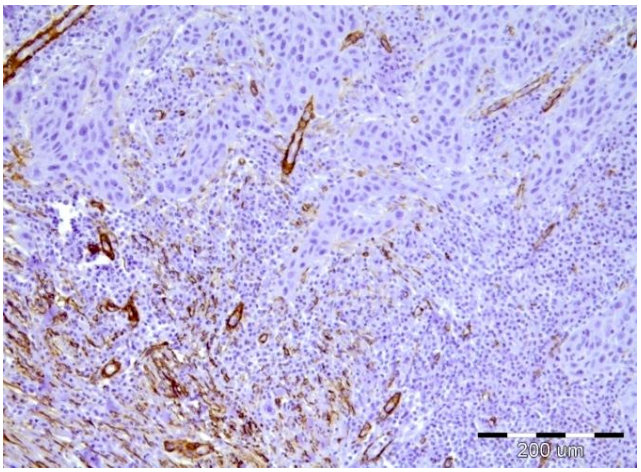

Figure 3: Vascular density with immunohistochemical staining for CD34 (10 x 4)

The analysis of histopathological sections was made with a light microscope. Also, a standard histopathological analysis was made of the surgical material from histopathological sections stained with hematoxylin-eosin. The histological results were analysed and presented in attributive and numerical statistical series. The results were evaluated with modern computer methods and software package and

tests for statistical processing: Kruskal Wallis test, Mann Whitney *U* test and Pearson's chi-square test.

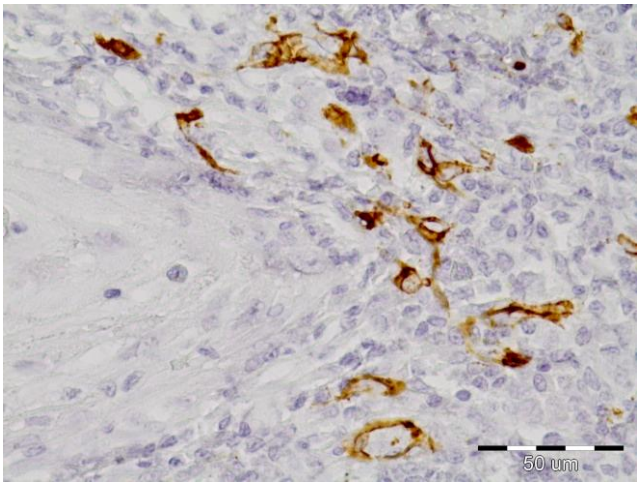

Figure 4: Visualization of vascular areas with immunohistochemical staining for CD34 at invasive front (10 x 100) with clearly formed lumina

Results

In this study, there were 13 out of 60 (21.7%) female patients, with age from 65 to 75 years, mean 70.2 ± 2.9 years and 47 (78.3%) male patients with age from 41 to 78 years, mean 66.4 ± 8.5 years.

All 15 patients with a metastatic deposit in the neck lymph nodes were men, aged 47 to 77 years, mean age 67 ± 8.2 years.

Regarding the grade of histological differentiation of SCC in patients with and without metastasis, the statistical analysis of data by applying the Pearson's chi-square test showed a highly significant statistical difference (p = 0.0000).

Figure 5 illustrates that the largest number of SCC in patients with metastasis were with poor histological differentiation. In patients without metastasis, SCCs with well histological differentiation were prevalent.

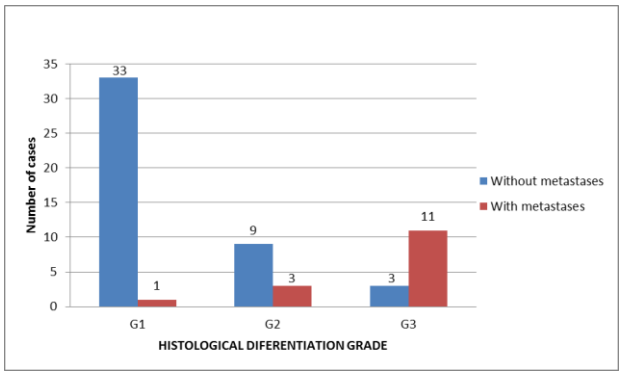

Figure 5: Comparison of the number of tumours with and without metastasis in the three groups with different grade of neoplasm differentiation (G1, G2, G3)

The lowest vascular density (smallest mean value) at the invasive front of the neoplasm was found in the well-differentiated SCC of the lower lip, and the highest in the poorly differentiated SCC of the lower lip.

Table 1: Vascular density at the invasive front of lower lip SCC in 60 patients about the grade of histological neoplasm differentiation (G)

Density of vascularization	Number of cases	Number of blood vessels			
		Mean value	Minimum value	Maximum value	Standard deviation
SCC of the lower lip					
G1	34	20.37	10.60	32.20	4.36
G2	12	24.90	18.60	37.30	6.27
G3	14	37.17	20.10	46.30	6.90

The mean values of vascularisation ranged from 20.4 to 37.2 (Table 1) and showed a statistically significant difference in the density of neovascularisation between groups of carcinoma with different grade of differentiation (Kruskal-Wallis test: H (2, N = 60) = 30.0943, p = 0.00001) (Figure 6).

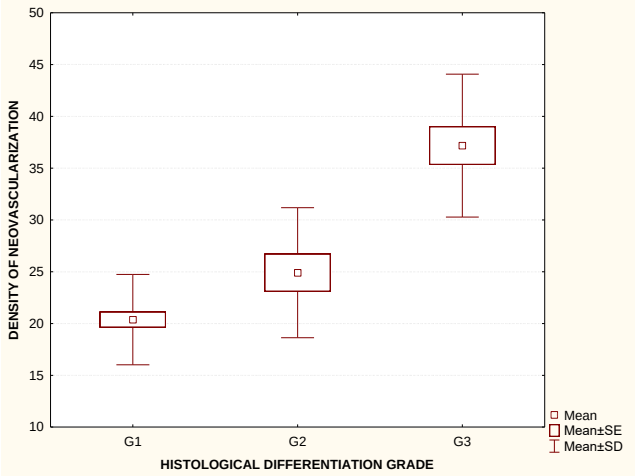

Figure 6: Statistically significant difference in the density of neovascularisation in lower lip SCC in 60 patients according to histological differentiation grade (G1, G2, G3). Legend: (G1) – well-differentiated carcinoma, (G2) – moderately differentiated carcinoma, (G3) – poorly differentiated carcinoma

Table 2 present the values of neovascularisation density in SCC and the surrounding non- tumour tissue.

Table 2: Vascular density in the invasive front of low lip SCC and surrounding non-tumour tissue

Density of vascularisation	No of cases	Mean value	Minimum value	Maximum value	Standard deviation
Non tumor tissue	60	6.4	4.70	8.70	0.97
SCC	60	25.20	10.60	46.30	8.71

The difference between the mean values of the vascular density in a tumour and non-tumour tissue was statistically significant (Mann-Whitney U тест-z = 9.447550, p = 0.0000001).

Table 3: Density of neovascularization in patients with neck metastasis from primary SCC of lower lip according to the grade of differentiation

Parameter	Number of cases	Number of blood vessels			
		Mean value	Standard deviation	Minimum value	Maximum value
G1	1	17.40	/	17.40	17.40
G2	3	27.13	9.08	19.80	37.30
G3	11	37.26	7.64	20.10	46.30

Table 3 and 4 present the values of neovascularisation (number of counted vascular spaces) at the invasive neoplasm front in patients with and without neck metastasis.

Table 4: Density of neovascularisation in patients without neck metastasis from primary SCC of lower lip according to the grade of differentiation

Parameter	Number of cases	Number of blood vessels			
		Mean value	Standard deviation	Minimum value	Maximum value
G1	33	20.46	4.39	10.60	32.20
G2	9	24.16	5.57	18.60	36.70
G3	3	36.86	4.17	32.10	39.90

The statistical analysis showed a significant difference in the density of vascularisation of lower lip SCC between neoplasms of patients without metastasis and neoplasms of patients with neck metastasis (Mann-Whitney U test, p = 0.000198) (Figure 7).

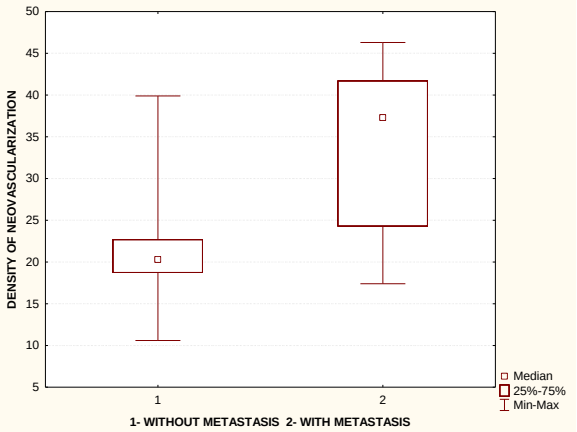

Figure 7: Statistically significant difference between vascular density in lower lip SCC in neoplasms without metastasis and neoplasms with metastasis

Discussion

Oral squamous cell carcinoma accounts for 95% of all forms of carcinomas that appear in the region of the head and neck. Squamous cell carcinoma is the most common carcinoma of the oral cavity, and the lower lip carcinoma is the most common carcinoma in the oral cavity [12]. Metastatic deposits in the regional lymph nodes are a predictive and prognostic parameter in patients with squamous cell carcinoma of the lower lip [13].

Metastases are less frequent in patients with a well-differentiated tumour (about 5%) than in patients with undifferentiated carcinomas (20%). Early detection of this carcinoma is important for obtaining esthetic and functional postoperative results and for favourable prognosis in patients [7].

Prognosis of squamous cell carcinoma of the lower lip depends on several factors, which include exposure to various risk factors and their combinations [4], stage of the disease [14], size of the tumor [15], presence of metastasis in the regional lymph nodes, different histological variables such as tumor grade, maximal thickness, perineural invasion and proteins expression [16], as well as other factors including surgical criteria [4]. The microvascular density of neoplasms is one of the histological elements that have been investigated in squamous cell carcinoma, but also other malignant neoplasms, aimed at early prevention of metastasis development and poor prognosis in patients with malignancies [17].

It has been determined that microvascular density (MVD) in tumours from the oral cavity does not differ significantly between oral mucosa and epithelial dysplasia, but is significantly increased in the tumour tissue. Also, it has been demonstrated that there is no expression of angiogenetic factors in the oral mucosa, but it is expressed in the tumour tissue and is in correlation with microvascular density, T status, tumour differentiation and grade of invasion [18]. Several studies that have examined MVD in a tumour have shown that it is higher in the tumour tissue than in the controls, it is a risk factor for the onset of metastasis [19], is in correlation with the grade of differentiation (G) and the onset of nodal metastasis [10]. Some authors consider that neoangiogenesis happens in the early phase of the development and its density is parallel to tumour progression [8].

Li C at al., reported that MVD was related to T status, stage of invasion and tumour differentiation as we also have found considering tumour differentiation [18]. Sedivy R at a.,l analyzing expression of vascular endothelial growth factor-C in correlation with MVD and the nodal status in oral SCC cell cancer reported that MVD (lymphatic and blood) results in a higher risk for cervical lymph node metastasis and that the angiogenetic effect of VEGF-C may also favour the onset of late lymphatic and haematogenous metastases [19]. These findings correspond to our findings that MDV predict the onset of neck nodal metastasis.

In this study, the density of neovascularisation was statistically significantly higher in carcinomas with a higher grade G3 of differentiation (poorly differentiated neoplasms) compared to those with a lower grade G1 (well-differentiated neoplasms) as well

as in patients with neck metastasis compared to those without neck metastasis.

In conclusion, the density of neoangiogenesis is increased in tumours with poorer differentiation and in patients with neck metastasis. So, the density of neovascularisation of the primary lip SCC may predict the tumour progression.

References

1. Moore SR, Johnson NW, Pierce AM, et al. The epidemiology of lip cancer: a review of global incidence and aetiology. Oral Dis. 1999; 5:185–195. https://doi.org/10.1111/j.1601-0825.1999.tb00300.x PMid:10483063

2. Khuder SA. Etiologic clues to lip cancer from epidemiologic studies on farmers. Scand J Work Environ Health. 1999; 25:125–130. https://doi.org/10.5271/sjweh.414 PMid:10360467

3. Maruccia M, Onesti MG, Parisi P, Cigna E, Troccola A, Scuderi N.Lip cancer: a 10-year retrospective epidemiological study. Anticancer Res. 2012; 32(4):1543-6. PMid:22493399

4. Moretti A, Vitullo F, Augurio A, Pacella A, Croce A. Surgical management of lip cancer. Acta Otorhinolaryngol Ital. 2011; 31(1):5–10. PMid:21808457 PMCid:PMC3146335

5. Agostini T, Spinelli G, Arcuri F, Perello R. Metastatic Squamous Cell Carcinoma of the Lower Lip: Analysis of the 5-Year Survival Rate. Arch Craniofac Surg. 2017; 18(2):105–111. https://doi.org/10.7181/acfs.2017.18.2.105 PMid:28913316 PMCid:PMC5556890

6. Vartanian JG, Carvalho AL, Araujo Filho, et al. Predictive factors and distribution of lymph node metastasis in lip cancer patients and their implications on the treatment of the neck. Oral Oncology. 2004; 40:223–227. https://doi.org/10.1016/j.oraloncology.2003.08.007 PMid:14693248

7. Zitsch RP. Carcinoma of the lip. Otolaryngol Clin North Am. 1993; 26:265–277. PMid:8460042

8. Florence ME, Massuda JY, Bröcker EB, Metze K, Cintra ML, Souza EM. Angiogenesis in the progression of cutaneous squamous cell carcinoma: an immunohistochemical study of endothelial markers. Clinics. 2011; 66(3):465-8. https://doi.org/10.1590/S1807-59322011000300018 PMid:21552674 PMCid:PMC3072009

9. Loggini B, Boldrini L, Gisfredi S, Ursino S, Camacci T, De Jeso K, Cervadoro G, Pingitore R, Barachini P, Leocata P, Fontanini G. CD34 microvessel density and VEGF expression in basal and squamous cell carcinoma. Pathol Res Pract. 2003; 199(11):705-712. https://doi.org/10.1078/0344-0338-00486 PMid:14708636

10. Ascani G, Balercia P, Messi M, Lupi L, Goteri G, Filosa A, Stramazzotti D, Pieramici T, Rubini C. Angiogenesis in oral squamous cell carcinoma. Acta Otorhinolaryngol Ital. 2005; 25(1)13-17. PMid:16080310 PMCid:PMC2639850

11. Shivamallappa SM, Venkatraman NT, Shreedhar B, Mohanty L, Shenoy S. Role of angiogenesis in oral squamous cell carcinoma development and metastasis: an immunohistochemical study. Int J Oral Sci. 2011; 3(4):216-224. https://doi.org/10.4248/IJOS11077 PMid:22010580 PMCid:PMC3469979

12. Taghavi N, Yazdi I. Prognostic factors of survival rate in oral squamous cell carcinoma: clinical, histologic, genetic and molecular concepts. Arch Iran Med. 2015; 18(5):314-319. PMid:25959914

13. Huang SH, Hwang D, Lockwood G, Goldstein DP, O'Sullivan B. Predictive value of tumor thickness for cervical lymph-node involvement in squamous cell carcinoma of the oral cavity: a meta-analysis of reported studies. Cancer. 2009; 115(7):1489-97. https://doi.org/10.1002/cncr.24161 PMid:19197973

14. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FI, Trotti A. Cancer staging handbook: from the AJCC cancer stading manual. AJCC editors, 8th edition. New York: Springer, 2017.

15. Luna-Ortiz K, Güemes-Meza A, Villavicencio-Valencia V, Mosqueda-Taylor A. Lip cancer experience in Mexico. An 11-year retrospective study. Oral Oncol. 2004; 40(10):992-999. https://doi.org/10.1016/j.oraloncology.2004.04.013 PMid:15509490

16. Rodolico V, Barresi E, Di Lorenzo R, Leonardi V, Napoli P, Rappa F, Di Bernardo C. Lymph node metastasis in lower lip squamous cell carcinoma in relation to tumour size, histologic variables and p27Kip1 protein expression. Oral Oncol. 2004; 40(1):92-8. https://doi.org/10.1016/S1368-8375(03)00141-6

17. Hollingsworth HC, Kohn EC, Steinberg SM, Rothenberg ML, Merino MJ. Tumor angiogenesis in advanced stage ovarian carcinoma. Am J Pathol. 1995; 147:33. PMid:7541612 PMCid:PMC1869875

18. Li C, Terakado N, Klosek SK, Ishikawa T, Nakashiro K, Hamakawa H. Microvessel density and expression of vascular endothelial growth factor, basic fibroblast growth factor, and platelet-derived endothelial growth factor in oral squamous cell carcinomas. Int J Oral Maxillofac Surg. 2003; 34(5):559-565. https://doi.org/10.1016/j.ijom.2004.10.016 PMid:16053878

19. Sedivy R, Beck-Mannagetta J, Haverkampf C, Battistutti W, Hönigschnabl S. Expression of vascular endothelial growth factor-C correlates with the lymphatic microvessel density and the nodal status in oral squamous cell cancer. Journal of oral pathology & medicine. 2003; 32(8):455-60. https://doi.org/10.1034/j.1600-0714.2003.00168.x