Original Article

Lymphatic metastasis in head and neck squamous cell carcinoma

Mohammad Hossein Baradaranfar[1], Shokouh Taghipour Zahir[2], Saeid Atighechi[3], Mohammad Hossien Dadgarnia[4], Fahimeh Bayati[5], Mohammad Reza Mohajerani[6]

ABSTRACT

Objective: Squamous cell carcinoma is the common type of malignancy in head and neck area and it metastasis into the regional lymphnodes. The objective of this study was to evaluate the detection of this metastasis via lymphatic channels around the tumor or via newly formed lymphatics inside the tumor.

Methodology: This case series included twenty patients specimens with head and neck squamous cell carcinoma. They were evaluated for lymphangiogenesis by using Vascular Endothelial Growth Factor 3 and KI67 immunohistochemical markers and then the data was correlated with clinicopatological criterias.

Results: High intratumoral and peritumoral lymphatic densities were both found significantly associated with the poor histological differentiation (Exact test p= 0.023). There was no association between intratumoral lymphatic density, peritumoral lymphatic density and the presence of lymph node metastasis, location of the tumor, age, and sex. There was however a significant association between intratumoral lymphatic density and peritumoral lymphatic density (Fisher exact test p=0.001).

Conclusions: This study reveals the existence of intratumoral and peritumoral proliferating lymphatics, but these lymphatics have no correlation with the lymph node metastasis in all the head and neck squamous cell carcinoma cases such as larynx and oral cavity.

KEY WORDS: Lymphangiogenesis, Squamous Cell Carcinoma, Intratumoral Lymphatic Density, Peritumoral Lymphatic Density.

Pak J Med Sci April - June 2011 Vol. 27 No. 2 303-306

How to cite this article:

Baradaranfar MH, Zahir ST, Atighechi S, Dadgarnia MH, Bayati F, Mohajerani MR.Lymphatic metastasis in head and neck squamous cell carcinoma. Pak J Med Sci 2011;27(2):303-306

INTRODUCTION

Head and Neck Squamous Cell Carcinoma (HNSCC) is a malignant neoplasm of squamous epithelium and is one of the most common head and neck malignancies. Tumor dissemination is often due to an invasion to lymphatic vessels. HNSCC is common in middle aged individuals and increases with age up to seventies and eighties. Male to female ratio is greater than 2:1.

The main factor that affects the treatment and prognosis of the disease is clinical staging of the tumor, and nodal metastasis is a main part of the staging system. Regardless of the site of the primary tumor, the presence of lymph node in either the ipsilateral or contralateral side of the neck reduces the 5-year survival by 50%.[1]

Spreading of the tumor may occur through, local tissue invasion. Direct seeding of body surfaces, hematogenous way or via lymphatic channels. Lymphatic metastasis is the most common pathway in spreading of the HNSCC and is also a main prognostic factor. Despite these well established principles,

Correspondence:

Shokouh Taghipour Zahir, MD, AP, CP,
Safayee, Ebnecina Blv, Shahid Sadoughi General Hospital,
Pathology Department, Yazd,
IRAN.
E-mail: staghipourzahir@yahoo.com

* Received for Publication: June 1, 2010

* Accepted: January 6, 2011

many key questions regarding the mechanisms of lymphatic tumor spread still remain unanswered. Is the spreading due to the formation of new lymphatic vessels inside of the tumor (lymphangiogenesis) or is it through the preexisting lymphatic vessels around the tumor.[2] There were controversies in literatures about this.[2-7] The detection of the new lymphatic vessels and the lymphangiogenesis inside the tumor can be evaluated by using specific immunohistochemical markers anti lymphatic vessels such as vascular endothelial growth factor 3(VEGFR3) and KI67 marker (for detection of proliferating endothelial cells) that distinguish them from blood vessels. Double staining with these two markers help us to discover the newly developed lymphoid vessels (lymphangiogenesis). Therefore this study was done to evaluate the amount of active lymphangiogenesis in HNSCC patients. If any active lymphangiogenesis was detected its relation with the existence of the nodal metastasis at the time of diagnosis and other clinicopathologic criteria were also investigated.

METHODOLOGY

Twenty patients specimens (paraffin embedded blocks) which had a HNSCC taken from the pathology ward were included in this study. The median age of the patients was 59.7 (range 36-81) years. Fourteen of the patients (70%) were male and 6 (30%) were female. Seven patients (35%) presented lymph node metastasis at the time of diagnosis. Two of the patients (10%) had cancers classified as low (I, II), 18(90%) as high (III, IV) clinical stage. Ten of the patients (50%) had tumors with well differentiation, nine (45%) of the tumors were with moderate differentiation, and one (5%) was poorly differentiated. All of the included samples originated from complete resection specimens with sufficient amounts of normal tissue surrounding the invasive tumors. For detection of lymphangiogenesis VEGFR3 was used to highlight lymphatic vessels and the monoclonal antibody KI67 was used for the detection of proliferating endothelial cells.

Double staining for VEGFR3/KI67 was performed in all tissue samples, using *STREPAVIDIN_BIOTIN* procedure as described later. (Figure 1) After staining, all the slides were examined by pathologist without knowledge of the clinical data. Then, the lymphatic density was counted using ×40 magnification per hpf. The Counts were performed both within the tumor area (intratumoral lymphatic density; ILD) and within an area 500ìm from the tumor border (peritumoral lymphatic density; PLD) as described before. For statistical analysis; the mean value of the above measurements was used as a cut-off point to separate tumors with high versus low lymphatic density. We also considered whether there was invasion of the VEGFR3/KI67 stained intratumoral or peritumoral lymphatic vessels by cancer cells as described by Kyzas.[7] The information was analysed by using SPSS software version 11. To compare ILD and PLD with clinicopathological variables, we used fisher exact test and exact test, and etc, as appropriate. All differences were considered significant if $p<0.05$.

RESULTS

The mean Intratumoral lymphatic density (ILD) in this study was 17 with a range of (1-50). Ten (50%) patients were included in the group with high ILD and 10 (50%) in the group with low ILD. The mean PLD was 3.5 with a range of 2-10. Similar to ILD, 10(50%) were in the group with high PLD and 10(50%) in the group with low PLD. Higher ILD and PLD correlated with the poor histological differentiation (Exact test p=0.023). There was no significant correlation between ILD and PLD on one hand and the presence of lymph node metastasis on the other at the time of diagnosis. Also, there was no association between ILD and PLD on one hand and the location of the tumor (larynx and oral cavity), age and sex on the other. Higher clinical stage had higher ILD and PLD but failed to attain statistical significance (Exact test p=0.17).

There was a significant correlation between ILD and PLD (Fisher exact test p=0.001).There was no tumor cells (emboli) within intratumoral or peritumoral proliferating lymphatics.

DISCUSSION

Squamous cell carcinoma is the main factor of morbidity and mortality. Many of the patients can be treated with surgical resection as long as the neoplasm is limited to its original site. Unfortunately most cancers lead to metastasis to other parts of the body which ends in patient's death. In general, cancerous cells can spread in many ways such as local invasion, hematogenous, and through lymphatic system. Infiltration of lymphatic vessels by tumoral cells is found around the tumors and the lymphatic system acts as spreading paths for the tumoral cells.[2] It is known that involvement of the lymphatic vessels at the time of diagnosis is one of the main prognostic factors in patients with HNSCC; it affects the treatment options.[8] Therefore, the specifications of the original tumor that can predict the nodal metastasis are very important.[9] In this study, we selected 20 patients with HNSCC

and different clinicopathological conditions in terms of the nodal metastasis, clinical stage, etc. Then we stained the paraffinic blocks with immunohistochemical method (IHC) and tried to highlight the lymphatic vessel proliferation (active lymphangiogenesis) inside and around the tumors.

In a previous study the lymphatic proliferation has been detected with a double staining technique using KI67/LYVE1.[5] But LYVE1 has been also seen in endothelium of the blood vessels.[3,10] In another study Podoplanin was used as a marker for the lymphatic endothelium and it was shown to be lymphatic specific.[7]

Since we could not find these markers in our country (for IHC staining), we used VEGFR3 which is another marker for the endothelium of the lymphatic vessels. In some of the previous studies it was mentioned that this marker can be found in small new blood vessels and also in the blood vessels of the tumoral cells.[2,6,10] But in some other studies the VEGFR3 marker was shown to be specific for staining.[3] Therefore we considered specifications of the lymphatic vessels to distinguish them from any blood vessels that might have been colored as well. It must be kept in mind that the lymphatic vessels are characterized by thin walls with irregular shapes that usually consist of 2-3 endothelial cells. In addition to the above factors, existence of blood cells helped us to distinguish the blood vessels from the lymphatic vessels. We used double staining with VEGFR3/KI67.

In order to have a completely blind study, the pathologist studied the samples that did not include any patient's information; only the investigator had access to patient's data. It was previously believed

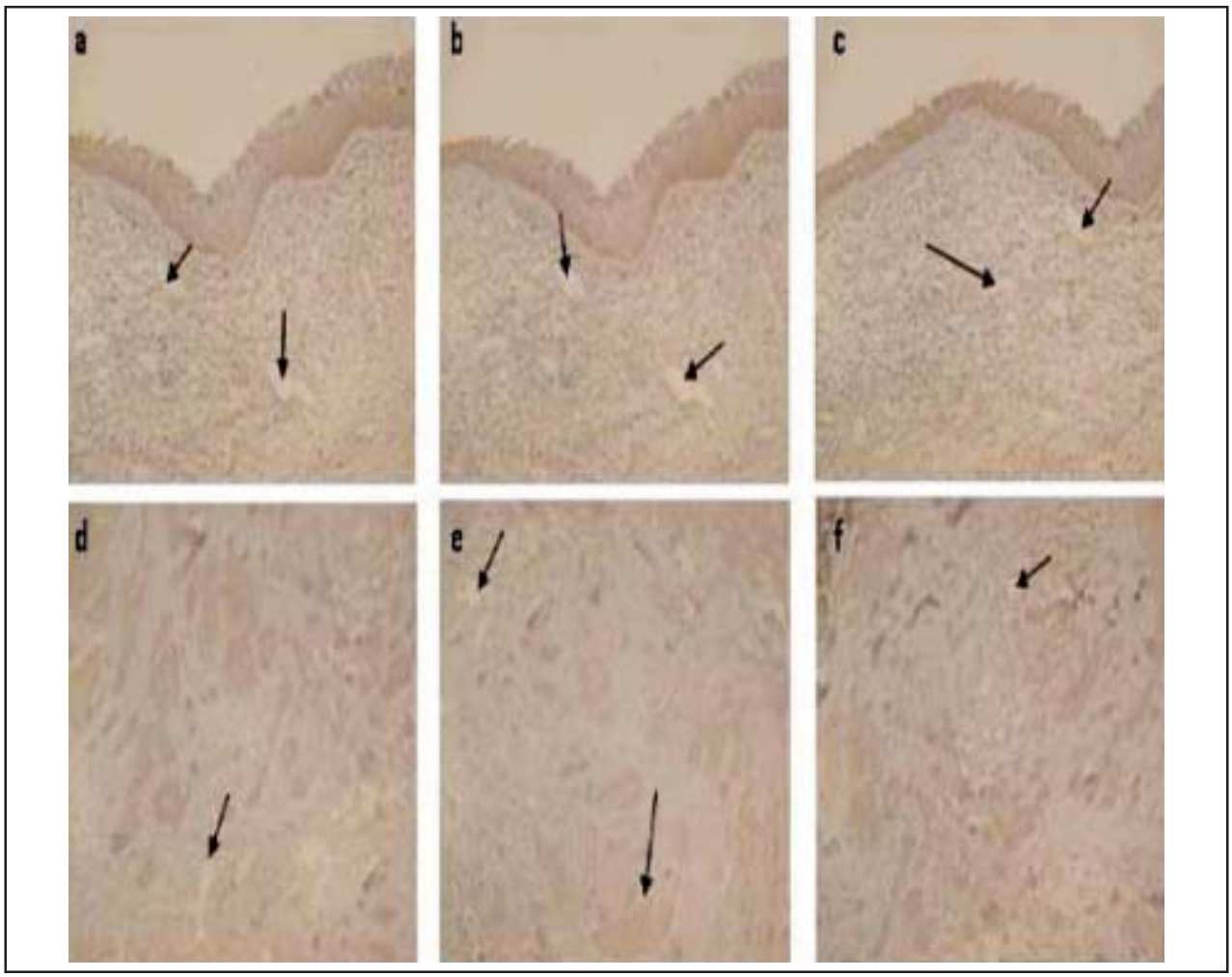

Figure-1: Peritumoral VEGFR3/k167-Positive lymphatic vessels (a, b, c), Intratumoral VEGFR3/k167-Positive lymphatic vessels (d, e, f). Black arrows shows VEGFR3/k167-Positive lymphatic vessels

that intratumoral lymphatics are non-functional and peritumoral lymphatics are enough for the metastasis. But new studies have shown that these intratumoral lymphatics can affect the nodal metastasis and can be an important indicator for poor prognosis.[5-7,11] Based on this fact, similar to Kyzas et al, we have studied both of proliferating lymphatics inside the tumor and also excistance or not of the tumoral cells inside the newly lymphatic channels. Although our study like Beasly et al, did not find any tumoral cell inside the new lymphatic vessels, Kyzas' study showed a few of tumoral cells in new lymphatics with significant correlation with nodal metastasis. This phenomenon may be explained by the fact that there are limitations in detecting what may be a rare event by examining small sections of archival tissue at a single point in time.[5,7]

In our study, tumoral differentiation had a significant relation with ILD and PLD which was similar to a previous study.[7] In contrast with some studies we did not find any significant relation between the nodal metastasis and lymphangiogenesis inside and around the tumor.[7,12,13] In a study by Beasly et al, oropharyngeal carcinoma was significantly correlated with neck node metastasis but larynx and oral cavity displayed no correlation between ILD and lymph node metastasis. The sites of tumors that we investigated were larynx and oral cavity and our results in these sites were similar to Beasly's study. In this and other studies HNSCC were lymphangiogenic but they varied in capacity to invade lymphatics.[5,7,12,13] This could be have another reasons such as increase vessel permeability due to different production or activation of factors such as matrix metalloproteinases, (transforming growth factor ß) TGFß or (vessel endothelial growth factor α)VEGFα.[5]

CONCLUSION

In this study, there is a significant association between ILD and PLD and our results suggest that although proliferating lymphatics can occur in HNSCC, they don't contribute to lymph node metastasis in all cases. It requires further testing on factors which contribute to increasing lymphatic permeability and invasion.

Ethics: Data were extracted from residency research dissertation that was approved by Shahid Sadoughi University of Medical Sciences Research Center.

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Authors:

1. Mohammad Hossein Baradaranfar, Associate Professor, Department of Otolaryngology.
2. Shokouh Taghipour Zahir, Assistant Professor of Clinical and Surgical Pathology, Department of Pathology,
3. Saeid Atighechi, Assistant Professor, Department of Otolaryngology.
4. Mohammad Hossien Dadgarnia, Assisstant Professor, Department of Otolaryngology.
5. Fahimeh Bayati, Otolaryngologist.
6. Mohammad Reza Mohajerani, Assistant Professor of Surgical and Clinical Pathology, Department of Pathology.

1-4: Shahid Sadoughi University of Medical Sciences, Iran.
5, 6: Esfahan University of Medical Sciences, Iran.