Original Research

Comparison of Tumor Infiltrating Lymphocyte Density with Histopathological Parameters and Effect on Prognosis in Head and Neck Squamous Cell Cancers

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Abstract

Objectives: The study aims to determine the ratio of the tumor-infiltrating lymphocytes (TIL) in the tumor microenvironment in squamous cell carcinoma of head and neck and its effect on prognosis using histopathological parameters.

Methods: The patients who underwent head and neck surgery with the diagnosis of squamous cell carcinoma of head and neck at Cerrahpaşa medical faculty ENT Clinic between January 2010 and November 2013 were included in this study. The age, gender, smoking status, alcohol use, radiologic images, and operation technique were analyzed for all patients. TNM pathologic staging, histologic differentiation grade, desmoplastic stromal reaction, vascular and perineural invasion, and lymph node metastasis were also evaluated. Representative hematoxylin-eosin stained slides from each block were cut and the ratio of tumor-infiltrating lymphocytes in tumor tissue was examined by an expert to confirm histology.

Results: In this study, 114 patients (105 males and 9 females) met inclusion criteria and were included. The mean age was 60.3±9.7 (ranging from 27 to 85 years). TIL and desmoplastic stromal reaction were compared statistically to the extent of primary tumor, vascular and perineural invasion, lymph node metastasis and histological grade of the tumor. While there was no statistically significant difference between TIL and these parameters, there was a statistically significant correlation between desmoplastic stromal reaction and these parameters. Considering five years of patient survival, although TIL had a positive impact on the prognosis of the tumor, there was no statistically significant difference.

Conclusion: We suggest that besides TNM pathologic staging and histologic parameters, immune cells reacting to the tumor will be a distinctive factor in determining the prognosis and new treatment methods. We believe that TIL will affect the current cancer treatments by increasing its anti-tumor effects and will give promising results in cancer immunotherapy.

Keywords: Desmoplastic stromal reaction; head and neck squamous cell carcinoma; histological parameters; tumor-infiltrating lymphocytes.

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Cancers occurring in the oral cavity, salivary glands, nasal cavity and paranasal sinuses, pharynx, larynx and lymph nodes in the upper part of the neck are called head and neck cancers. Although head and neck cancers rank lower than other types of cancer concerning their incidence and mortality, they are of particular importance due to the anatomical and functional characteristics of their location.[1]

Despite major advances in multidisciplinary treatment regimens for this important disease, how to improve long-term survival remains an indisputable clinical issue. There is a need to identify more specific biomarkers in order to optimize the classification of patients and the choice of subsequent therapy and to develop more effective therapeutic approaches to treat head and neck cancers. In particular, cancer immunotherapy has altered existing cancer therapies by stimulating or activating the host anti-tumor immune response, and has shown durable and promising results in various cancers.[2]

The determinative effects of biological factors, such as DNA content, immunological status of the tumor and circulating immune complexes on life expectancy, are still under investigation.

The immune system produces antibodies against bacteria and viruses because it perceives the infection as a danger signal. While tumor cells recognized by the immune system are most likely destroyed, cells that manage to hide from the immune system survive. Tumor-associated antigens are considered by the body as its own antigens, and the immune response to these antigens remains insufficient. Tumor cells that manage to escape from the immune system and adapt to the microenvironment become resistant to immune system cells. Another obstacle to the development of an effective immune response against the tumor is some immunosuppressive factors that the tumor releases.[3]

Based on this relationship between tumor cells and the immune system, Burnett et al.[4] proposed the idea of immune surveillance in 1960 and suggested that tumor cells are recognized by the immune system. Today's modern immune surveillance theory emphasizes that a person's immune system has the ability to detect and destroy tumor cells. In addition, this theory argues that tumor cells are not passive targets for the immune system but have the ability to escape and inactivate a person's immune system. This theory expresses the complexity of the interactions between tumor cells and cells of the immune system or their products. As a result of these interactions, it is estimated that the immune system cell, not the tumor cell, often dies.[2]

It was noticed long years ago that lymphocytes, which are among the immune system cells, were in different numbers in malignant tumors. These lymphocytes were later defined as tumor-infiltrating lymphocytes (TILs).[5] TILs include antigen-specific B cells, Natural killer (NK) cells, adaptive immune effector cells and immunosuppressive cells and responsible for tumor regression by killing the tumor cell.[6] At first, these TILs were thought to indicate chronic inflammation in cancer; then it was discussed whether TILs created a good environment for cancer growth or emerged as an immune response against cancer.[5] The relationship between prognosis and immune cell infiltration was first established on breast cancer cases in 1949.[7] Later, this finding was shown to have prognostic significance.

In advanced stages of tumors, such as colon, breast, head and neck cancers, the presence of TIL in the tumor area, has been shown to increase the life expectancy of the patient.[8]

The effect of TILs on prognosis in different tumors has been evaluated in many studies. This study was planned to reveal the importance of TILs density and desmoplastic stromal reaction in head and neck cancers. We aimed to compare the density of TILs with histopathological parameters in head and neck squamous cell cancers and to evaluate its effects on prognosis and whether it can be used as a new prognostic marker in patients with head and neck cancers.

Methods

In this retrospective study, 114 patients who underwent head and neck surgery with the diagnosis of head and neck cancer at Cerrahpaşa medical faculty ENT Clinic between January 2010 and November 2013 were included. The tumors were staged according to the American Joint Committee on Cancer 2010 criteria. Age, gender, alcohol and smoking habits of the patients and tumor localization were recorded. Size of the tumor (T 1, 2, 3, 4), histological differentiation (well, moderate, poor), lymphocyte infiltration density showing immune response against tumor (mild: 1, moderate: 2, strong: 3), stromal reaction (weak: 1, medium: 2, strong: 3), presence or absence of vessel, perineural and lymphovascular invasions, presence or absence of lymph node metastasis and perinodal extension, were evaluated. Local ethics committee approval was obtained for this study (Date: 06.04.2014, ethics committee decision 83045809/604 / 02-6027).

Evaluation of Intratumoral Inflammation

Pathological preparations were stained using Hematoxylin-Eosin, and sections were cut into paraffin blocks. All available sections for each patient were examined and the sections with the highest TIL density were selected. Adenoma, ulceration and necrotic areas were excluded. Tumor-infiltrating lymphocytes (TIL) density was calculated

as the ratio of the area occupied by mononuclear cell infiltrates over the entire stromal area (%TIL = area occupied by mononuclear cells in the tumor stroma/total stromal area). TIL density was evaluated at ×100 and ×400 magnification and categorized as mild (<30%), moderate (>30% and <60%) and strong (>60%).

Statistical Analysis

"SPSS 21.0" statistical program was used for the statistical evaluation of the collected data. Pearson chi-square test (x^2) and Pearson correlation analysis (r coefficient) were used in the evaluation.

Mean±standard deviation and median (minimum, maximum) values were determined for the results. In evaluating whether the difference between results in relationships is statistically significant or not, a 'p' value less than 0.05 was considered statistically significant.

Results

Of the 114 patients included in this study, 105 were male (92.1%) and nine were female (7.9%). The patients were minimum 27 and maximum 85 years old, with a mean age of 60.3±9.7. 105 of the patients had the smoking habit and 20 were alcohol users. Non-smoker was defined as '0' pack/year, the highest smoking rate as 144 packs/year, with a mean value of 43±28 packs. When 114 patients included in this study were evaluated according to the primary tumor location, laryngeal cancer was the majority with 97 patients (85.1%). 10 (8.8%) of the other 17 patients had oral cavity cancer and seven (6.1%) had oropharyngeal cancer (Table 1).

When evaluated according to tumor size, nine patients (7.9%) were T1, 24 patients (21.1%) were T2, 32 patients (28.1%) were T3 and 49 patients (43.0%) were T4. When evaluated according to lymph node metastasis, 66 patients (57.9%) were N0, 13 patients (11.4%) were N1, 35 patients (30.7%) were N2; and among the N2s, one patient was N2a, 16 patients were N2b and 18 patients were N2c. None of the patients were evaluated as N3. None of the patients had distant metastases at the time of diagnosis. When evaluated according to their stages, seven of the patients were stage I (6.1%), 16 were stage II (14%), 20 were stage III (17.5%) and 71 were stage IV (62.3%). Patients with a histopathology of squamous cell carcinoma were included in the study group. According to the pathology results, three patients (2.6%) were grade 1, 56 patients (49.1%) were grade 2 and 55 patients (48.2%) were grade 3 (Table 2).

Blood vessel invasion was detected in 71 patients (62.3%) and no invasion was detected in 43 patients (37.7%). Lymphatic invasion was detected in 101 patients (88.6%), while 13 patients (11.4%) had no invasion. Perineural invasion was detected in 80 patients (70.2%), while there was no perineural invasion in 34 patients (29.8%). Perinodal extension was not detected in 92 patients (80.7%), while 22 patients (19.3%) had perinodal extension (Table 3).

When the density of TILs was examined, it was noted that as mild in 18 patients, as moderate in 64 patients, and as strong in 32 patients. Regarding the desmoplastic stromal reaction, 19 (16.7%) patients had weak, 70 (61.4%) had medium and 25 (21.9%) had strong desmoplastic reaction (Table 4).

Table 1. Patient characteristics

Total number of patients	114
Gender	
Female	9
Male	105
Mean age	60.3±9.7
Localization	
Larynx	97
Oral cavity	10
Oropharynx	7
Smoking	
Yes	105
No	9
Alcohol consumption	
Yes	20
No	94

Table 2. Histopathological characteristics of the tumor 1

	0	1	2	3	4
T		9	24	32	49
N	66	13	35	0	
Stage		7	16	20	71
Grade		3	56	55	

Tablo 3. Histopathological characteristics of the tumor 2

	Blood vessel invasion	Lymphatic invasion	Perineural invasion	Perinodal extension
Yes	71	101	80	22
No	43	13	34	92

Tablo 4. Histopathological characteristics of the tumor 3

	Tumor-infiltrating lymphocytes	Desmoplastic stromal reaction
Weak	18	19
Moderate	64	70
Strong	32	25

TIL density was compared with tumor size, lymphatic invasion, blood vessel invasion, perineural invasion, lymph node metastasis and perinodal extension, and no statistically significant difference was found between them. When the relationship with histological grade was examined, the findings showed that the decrease in TIL density (Pearson's R: 0.083) and desmoplastic stromal reaction (Pearson's R: 0.019) was significantly lower in poorly differentiated tumors than well-differentiated tumors, but no statistically significant difference was found. Compared with the stage, a low level of significant decrease in the density of TILs (Pearson's R: -0.101) was detected in advanced stage tumors, but no statistically significant difference was found.

When desmoplastic stromal reaction was compared with tumor size (p=0.014), lymphatic invasion (p=0.000), blood vessel invasion (p=0.037), perineural invasion (p=0.002) and stage (p=0.006), statistically significant results were found between them. Thus, as the tumor stage and spread progressed, desmoplastic stromal reaction against the tumor increased. This situation shows that with the increase in tumor cell density, the immunological response to the tumor becomes evident (Table 5).

Considering the 5-year survival rates of the patients, although TILdensity and desmoplastic stromal reaction had a positive effect on prognosis, no statistically significant difference was found.

Discussion

The relationship between cancer development and chronic inflammation has been discussed for many years.[9] The majority of inflammatory cells, such as neutrophils, monocytes, macrophages, eosinophils, mast cells and lymphocytes, accumulate during injury and infection, and may contribute to the formation, progression or regression of cancer.[9, 10]

It has been reported that lymphoplasmacytic infiltration surrounding the tumor stroma shows an immune response that develops against the tumor.[11] High density of TILs representing a potent intratumoral inflammatory response is considered a good prognostic marker in many malignancies,

such as breast cancer,[12] colon cancer[13] and gastric cancer.[14] In addition, it has been shown that high levels of intratumoral immune cell infiltrates in head and neck squamous cell carcinomas are associated with superior patient survival.[15-18]

There are also publications reporting that lymphoplasmacytic infiltration negatively affects survival. Scott et al.[19] showed in their series of 106 cases of inoperable non-small cell lung tumors that the systemic inflammatory effect reduced the patient's quality of life and negatively affected the prognosis.

Increase of inflammatory cells in tumoral tissue and surrounding normal parenchymal tissue has also been found to be associated with increased tumor grade and aggressive course. According to Kolbeck et al.'s small study with 24 cases, increased lymphocytic cell infiltration in the tumor increased the recurrence of the tumor.[20]

Lamb et al.[21] found no relationship between local lymphocytic infiltration and survival in these tumors.

Inflammation has been seen as a feature of carcinogenesis and affects many malignancy aspects, including the occurrence, progression and spread of cancer.[22] TILs were thought to indicate chronic inflammation in cancer, and then it was discussed whether they created a good environment for cancer growth or emerged as an immune response against cancer.[5] In a study, it was reported that laryngeal squamous cell cancer patients had a marked increase in inflammatory response when compared with chronic hypertrophic laryngitis and normal controls.[23]

In squamous cell cancers of the larynx, the infiltration of immune cells, such as mast cells, neutrophils and macrophages can produce small molecules, such as cytokines, chemokines and growth factors that support carcinogenesis and tumor angiogenesis. It activates tumors to avoid the host immune response as well as produce more immune cells.[24] In addition, squamous cell carcinomas themselves can overexpress cytokines with their proinflammatory, proangiogenic and immunoregulatory activity.[23, 25]

Studies on head and neck cancers have generally found that lymphoplasmacytic infiltration and stromal response

Table 5. The relationship of stromal reaction with histopathological parameters

Desmoplastic stromal reaction	T				Lymphatic invasion		Blood vessel invasion		Perineural invasion		Stage			
	1	2	3	4	Yes	No	Yes	No	Yes	No	1	2	3	4
Weak	5	3	6	5	10	9	7	12	7	12	4	3	6	6
Moderate	3	19	17	31	67	3	46	24	53	17	2	12	8	48
Strong	1	12	9	13	24	1	18	7	20	5	1	1	6	17
P		0.014			0.000		0.037		0.002		0.006			

are associated with survival, but there is no clear information on this subject. Shuichi Kawashirive et al. investigated the importance of stromal desmoplasia and appearance of myofibroblasts in anterior oral cavity squamous cell cancers and showed that the appearance of myofibroblast increased with tumor invasiveness and lymph node metastasis occurred more frequently in the myofibroblast-positive group, and the 5-year survival rate was significantly worse in this group.[26]

Vassilakopoulou et al.[16] reported that increased TIL density in laryngeal squamous cell carcinomas was associated with higher survival. In our study, the relationship between TIL density and desmoplastic stromal reaction with patient survival was evaluated, and although it had a positive effect on prognosis, no statistically significant result was found. When we evaluated the relationship between desmoplastic stromal reaction and TIL density and the histological grade of the tumor, the decrease in TIL density (Pearson's R: -0.083) and desmoplastic stromal reaction (Pearson's R: -0.019) was found to be low level significant in poorly differentiated tumors, but no statistically significant difference was found. This may indicate that the stromal reaction is insufficient in invasive and low-grade tumors and cannot prevent tumor spread. This decrease in desmoplastic stromal reaction and TIL density in poorly differentiated tumors may be due to the tumor's immunological bypassing the defense system more easily or an independent mechanism taking part in this process.

Conclusion

In the light of all this information, we anticipate that besides clinical TNM staging, histopathological parameters should be evaluated in detail at the stage of diagnosis and treatment plan, patient selection should be made well in planning treatment, and immune cells against cancer will guide the prognosis and determination of new treatment models. Especially in cancer immunotherapy, we think that by increasing the anti-tumor effects of TILs and by awakening or activating the host anti-tumor immune response, existing cancer therapies will be changed and will give durable and promising results in various cancers.

Disclosures

Ethics Committee Approval: Istanbul University Cerrahpaşa Faculty of Medicine Ethics Committee (88931902/615).

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