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Tumor Infiltrating Lymphocytes in Different Stages of Malignant Melanoma and Correlation with Tumor Stage and Other Prognostic Factors: A Retrospective Multicenter Study

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Abstract

Background: Melanoma is one of the most immunogenic tumors that causes a significant immune response. Tumor infiltrating lymphocytes are an important part of this response. Tumor infiltrating lymphocytes are lymphocytes in close association with tumor cells that have infiltrated tumor nests. In this study, we attempt to evaluate the relationship of tumor infiltrating lymphocytes in malignant melanoma with histopathologic findings, tumor stage, and other prognostic factors.

Methods: This was a retrospective cross-sectional study. We re-evaluated patients' specimens and categorized the tumor infiltrating lymphocytes as grades 0, 1, 2, or 3 based on density and distribution of the infiltrating lymphocytes.

Results: We enrolled 111 patients with a mean age of 59.33±14.68 years, and a male to female ratio of 1.09. There was no evidence of tumor infiltrating lymphocytes in 17.1% of patients. The melanoma subtypes had the following tumor infiltrating lymphocyte grades: 1 (47.7%), 2 (28.8%), and 3 (6.3%). Cancer stage significantly decreased with increasing grade of tumor infiltrating lymphocyte (P<0.001). Although numerous histopathologic findings had a relationship with tumor infiltrating lymphocytes, only microsatellitosis had a significant relation after adjustments for melanoma stage (P< 0.001).

Conclusion: Increased density of tumor infiltrating lymphocytes can show a more effective immune response against melanoma. This response can limit cancer progression and result in tumor diagnosis at lower stages of the disease.

Keywords: Melanoma, Tumor infiltrating lymphocytes, Tumor stage, Immune response, Prognostic factor

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Introduction

Malignant melanoma has the highest morbidity and mortality among skin cancers.[1] Malignant melanoma shows an immunogenicity response in the body which results in the development of immunological interventions.[2] The body has an effective cancer immune surveillance system, which configures and destroys premalignant and malignant cells.[3] Thus, avoidance of this system can be considered another hallmark of cancer.[4] Interaction between tumor cells in a microenvironment of non-malignant cells and migratory hematopoietic cells can determine the outcome of a cancer.[5] Hence, the role of lymphocyte infiltration in cancer development is increasingly under study. In different cancers, tumor infiltrating lymphocytes (TIL) are recognized as a sign of antitumor immunity and associated with a better outcome. The quantity and quality of TIL are of paramount important in the impact of TIL on cancer outcome.[6]

Tumor infiltrating lymphocytes are lymphocytes closely associated with tumor cells that have infiltrated and disrupted tumor nests. These lymphocytes can mark the beginning of tumor regression, a process which may result in partial or complete loss of malignant cells. The prognostic significance of TIL in melanoma is controversial.[7,8] Overall, melanoma is been considered as an example of immunogenicity among human cancers. Probably, an immune response in melanoma cannot primarily prevent the cancer incidence; however, it can make a difference in disease outcomes by control of tumor progression.[9] Robust mechanisms are employed to escape the immune system in melanoma metastases.[10] Autologous TIL has been used in a novel therapy for metastatic melanoma with promising results.[11]

We investigated the presence and grade of TIL in malignant melanoma patients and evaluated its relationship with stage of cancer and different histopathologic findings in malignant melanoma.

Table 1. Tumor infiltrating lymphocyte (TIL) grades 1-3 determined by density and distribution of lymphocytes among the tumor cells.

TIL distribution	TIL density		
	Mild	Moderate	Marked
Focal	1	1	2
Multifocal	1	2	2
Diffuse	2	3	3

Materials and Methods

We conducted this retrospective cross-sectional investigation at the Cancer Institute of Imam Khomeini Hospital Complex and Razi Dermatology Hospital, Tehran, Iran. We gathered data from the records of all patients with melanoma in the previous two years. All biopsies were reevaluated and all specimens in the evaluation and analysis remained anonymous.

Patient's age and sex were noted for each

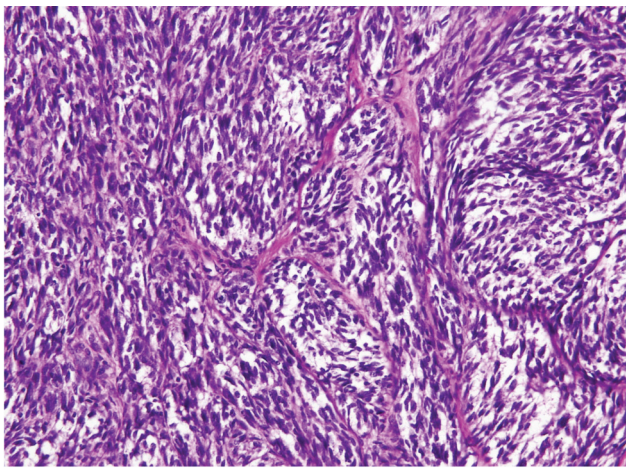

Figure 1. A. TIL grade 0: absent TIL infiltrate, (H&E, original magnification ×20)

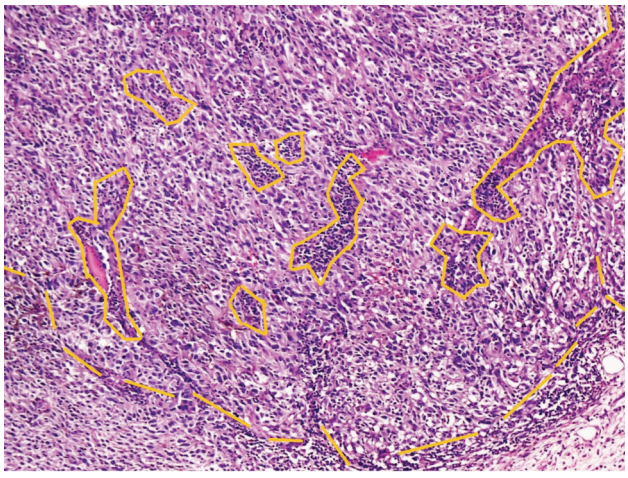

Figure 1. B. TIL grade 1: moderate focal TIL infiltrate in the center of tumor highlighted by yellow lines, (H&E, original magnification ×10).

Table 2. Age, male to female ratio, ulceration, microsatellitosis, vascular invasion, perineural invasion , metastasis , mitotic rate, and tumor thickness in 111 melanoma patients categorized by grade of tumor infiltrating lymphoctytes (TIL).

Property/TIL	Grade 0	Grade 1	Grade 2	Grade 3	Total	*P*-value	*P*-value after adjustment for stage
Age (years)	62.95	60.30	55.78	58.43	59.33	0.12	0.21
Male/female ratio	8/11	31/22	17/15	2/5	58/53	0.79	
Ulceration	13 (68.42%)	34 (64.15%)	13 (40.63%)	2 (28.57%)	62 (55.86%)	0.01	0.48
Metastasis	9 (47.37%)	16 (30.19%)	1 (3.03%)	1 (14.29%)	27 (24.32%)	0.00	0.87
Microsatellitosis	9 (47.37%)	14 (26.42%)	3 (9.68%)	0 (0%)	26 (23.42%)	0.00	0.01
Vascular invasion	7 (36.84%)	15 (28.30%)	5 (15.63%)	0 (0%)	27 (24.32%)	0.02	0.19
Perineural invasion	4 (21.05%)	10 (18.87%)	2 (6.25%)	0 (0%)	16 (7.58%)	0.05	0.43
Mitotic rate	4.26±2.60	3.79±3.5	2.71±1.47	2.29±1.25	3.47±2.58	0.01	0.07
Tumor thickness	11.39±9.64	9.96±15.37	5.03±6.80	1.87±1.17	8.27±12.20	0.01	0.21

specimen. The biopsy was placed in a subcategory of malignant melanoma: superficial spreading, lentiginous, acral, nodular or mucosal based on histopathologic criteria. We determined the melanoma stage according to the AJCC melanoma staging update. Lymphocyte infiltration in specimens was investigated. We categorized the degree of lymphocytic infiltration (TIL) based on the density and distribution of lymphocytes among the tumor cells into four grades as follows: grade 0 (no TIL infiltrate); grade 1 (mild or moderate focal or a mild multifocal TIL infiltrate); grade 2 (marked focal, either moderate or marked multifocal, or mild diffuse TIL infiltrate); and grade 3 (moderate or marked diffuse TIL infiltrate) as seen in Table 1 and Figure 1.

Data were entered in SPSS version 16 for further analysis. We used the logistic regression test to compare variables between different severities of lymphocyte infiltration. $P<0.05$ was considered significant for all tests.

Results

This study recruited 111 patients with a mean age of 59.33±14.68 years. There were 58 (52.3%) male and 53 (47.7%) female patients. The majority of patients (82.9%) had TIL. Most had TIL grade 1, followed by grades 2 and 3 (Figure 2).

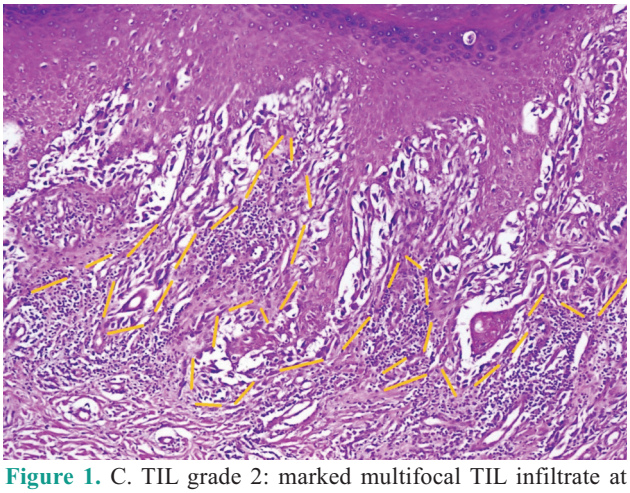

Figure 1. C. TIL grade 2: marked multifocal TIL infiltrate at dermoepidermal junction highlighted by yellow line (H&E, original magnification ×20).

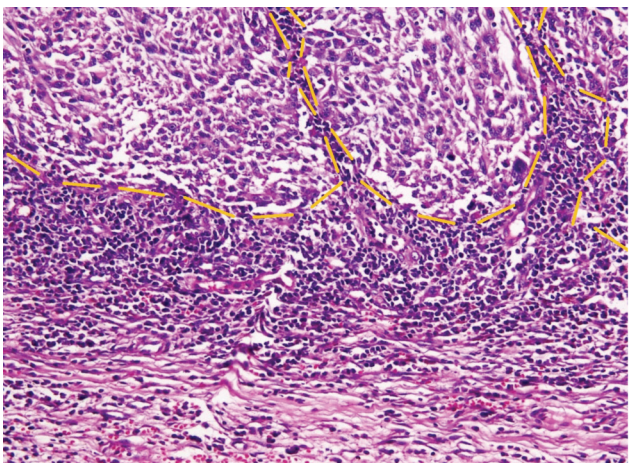

Figure 1. D. TIL grade 3: marked diffuse TIL infiltrate in invasive margin of the tumor highlighted by yellow line. (H&E, original magnification ×20).

Table 3. Clark level of invasion in 111 melanoma patients categorized by grade of tumor infiltrating lymphocytes (TIL).

TIL grade	Clark level					Total
	I	II	III	IV	V	
0	0	0	2	4	13	19
1	5	3	12	13	20	53
2	5	7	10	4	6	32
3	0	2	2	3	0	7
Total	10	12	26	24	39	111

The prevalence of melanoma subcategories was as follows: acral lentiginous (40.5%), nodular (35.1%), lentigo maligna (8.1%), superficial spreading (5.4%), and mucosal melanoma (10.8%). The most common severity of TIL in all melanoma types was grade 1, with the exception of lentigo melanoma which was grade 2 (Figure 3). There was no significant relation between melanoma type and TIL grade (*P*=0.72).

Table 2 shows demographic and histopathologic findings in melanoma patients categorized by degree of TIL. Age difference and male to female ratio did not significantly differ between different grades of TIL (*P*=0.35).

The prevalence of melanoma according to stage was: I (27.0%), II (50.5%), III (2.7%), and IV (19.8%). Figure 4 shows the prevalence of stage at the time of diagnosis for different grades of TIL. There were 47.37% of patients without TIL who had stage IV cancer, whereas no patients with TIL grade 3 had stage IV cancer. Stage of melanoma significantly decreased with increased grade of TIL (B: -0.54, *P*=0.00).

A total of 24.32% of patients had metastasis. Prevalence of metastasis was the highest in TIL grade 0 (47.37%). Metastasis significantly decreased with increasing grade of TIL (B: -1.12, *P*=0.002). These relationships were not significant after adjustment for cancer stage.

Microsatellitosis was observed in 23.9% of patients. A statistically significant difference existed with diminished rate of microsatellitosis and increased severity of TIL (B: -1.13, *P*=0.002). This relation remained significant after adjustment for stage (B: -0.95, *P*=0.01).

Tumor thickness was seen from the highest to lowest in grades 0-3 lymphocytic infiltrations. This difference was statistically significant (*P*= 0.01). After adjustment for stage, this relation was not significant.

Most patients with TIL grade 0 had Clark level invasion of V. Table 3 shows the prevalence of patients with different levels of Clark invasion. Decreased Clark level with TIL grade was statistically significant (B: -0.56, *P*= 0.001). After adjustment for stage, this relation was not significant (*P*=0.16).

Discussion

The presence of TIL has been reported for numerous tumors. This infiltration is a sign of the immune response to antigen tumors. The term "immunoediting" has been proposed to describe the events in the interaction of the immune system and tumor cells during the cancer course.[12] Immune cells in the tumor environment may be the body's response to tumor development. This lymphocyte may be recruited only because of tissue damage and inflammation.[13] The role of TIL in different cancers has been investigated. The first

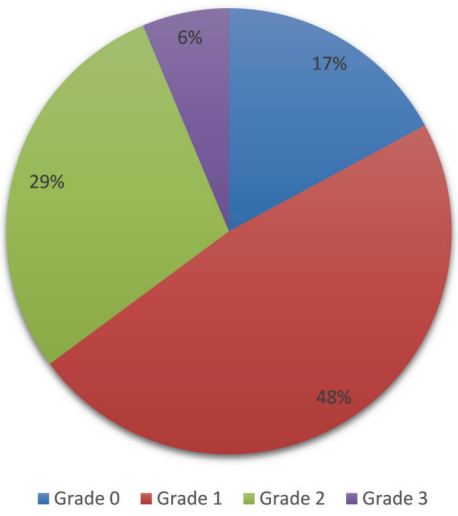

Figure 2. Frequency of different grades of TIL in in 111 melanoma patients.

article regarding melanoma to a better prognosis with TIL was published in 1981.[14]

In many cancers, infiltration by numerous lymphocytes seems to be associated with improved prognosis. Hence, the quantity of TIL may play an important role in cancer progression.[6] We have categorized TIL into four grades (0-3). Clark et al. suggested three categories for classification of TIL: absent, non-brisk, and brisk.[15] Another four system has been used in a study that considered both lymphocyte density and distribution.[7,16]

In our study most patients had TIL grades 0 or 1. This finding supported a study of the Australian melanoma database. In that study, 35.4% had no TIL and 45.1% had mild TIL.[7] Studies about role of TIL in malignant melanoma are controversial. Some studies have highlighted the role of TIL in thick or T4 melanoma.[17,18]

Effective treatment is possible with early diagnosis of melanoma at lower stages.[18] Advanced stages of melanoma with metastases will complicate treatment and necessitate further approaches.[19] In our study, we have observed a significant association between TIL and stage at the time of diagnosis. A large population-based study reported the association of non-brisk and brisk TILs with lower stage compared to no TILs. That study reported an association between TIL grade and decreased death, independent of tumor stage.[20] In another study on prognostic significance of TIL, the researchers found that TIL response was a major predictor of sentinel lymph node metastasis. However TIL did not significantly predict survival.[21] In another study, TIL was shown to be an independent predictor of both sentinel lymph node status and survival.[7] An investigation of nine clinical and pathological factors showed that TIL was one of the significant independent predictors.[22] It has been suggested that TIL might be a significant predictor of melanoma survival, but loses its importance when the sentinel lymph node status is known.[23]

In the current study, histopathologic findings such as number of mitoses, tumor thickness, and vascular and perineural invasion were associated with TIL. This was not a significant finding after adjustments for stage. However, the relation between low microsatellitosis and increased grade of TIL remained significant after adjustment for stage. Presence of microsatellitosis is an indicator of poor prognosis in malignant melanoma and associated with increased frequencies of ulcers and metastatic lymph nodes.[24,25]

Conclusion

Tumor infiltrating lymphocytes are a sign of an effective body immune response to tumor cells in melanoma. This response can limit further tumor progression. Limitations of tumor growth increase the chances for earlier detection of lower stage melanoma with less thickness, mitotic rate, perineural/vascular invasion and metastasis. Also TIL may have an independent effect on the presence of microsatellitosis at the time of diagnosis. These effects associated with TIL can contribute to the possibility of better management and increased survival.

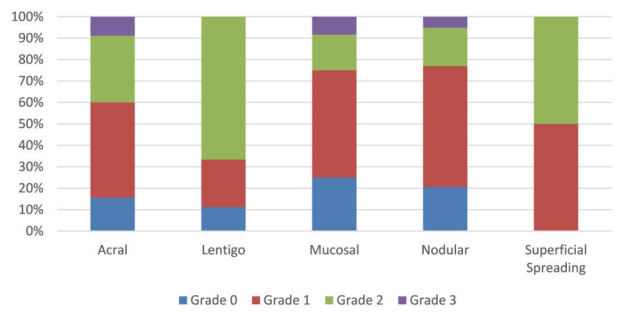

Figure 3. Grade of TIL in 111 melanoma patients categorized by type of melanoma.

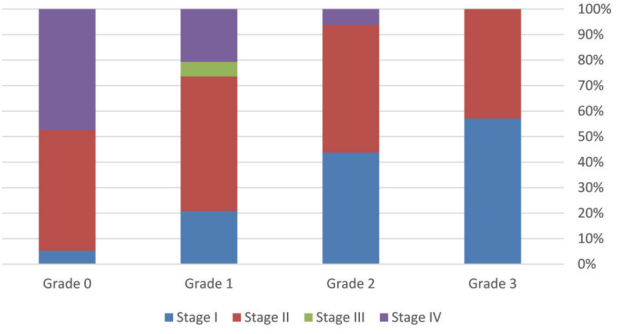

Figure 4. Stage of melanoma at time of diagnosis in 111 melanoma patients categorized by TIL grade.

Conflict of Interest

No conflict of interest is declared.

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