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THE ROLE OF INFILTRATION BY LANGERHANS CELLS IN THE ASSESSMENT OF MALIGNANT LARYNX TUMERS PROGNOSIS

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ABSTRACT

Background: Larynx cancer is a major challenge in ENT practice, so it requires deeper understanding, especially prognosis. Pathology in some studies on larynx cancer showed Langerhans cells infiltration in tumer stroma in some patients, which questions their role in prognosis assessment in these patients. **Objective:** To study the relation between langerhans cells infiltration on one side, and the malignant larynx tumers prognosis and other prognostic factors on the other side. **Patients and Methods:** A retrospective cohort study included patients whom biopsies from larynx tumers showed malignancy, and they had Laryngectomy in Tishreen University hospital between 2017 and 2020. **Results:** out of 21 cases, 16 were males (76.2%), 5 were females (23.8%), ages were between 41 and 76 years, age was converted to three groups, first from 41 to 50 years (23.8%), the second group from 51 to 60 years (28.6%), and the third group were above 60 years (47.6%), out of all cases 16 were smokers (76.2%), 4 were alcoholic (19%), Lymph node metastasis in 8 patients (38.1%), using s-100 immunostaining and morphology, minimum Langerhans infiltration was seen in 8 patients (38.1%), intermediate infiltration in 8 patients (38.1%), important infiltration in 3 patients (14.3%), and no infiltration in 2 (9.5%), and using CD1a immunostaining minimum infiltration was seen in 11 patients (52.4%), intermediate infiltration in 6 (28.6%), important infiltration in 2 (9.5%) and no infiltration in w (9.5%). the study showed statically important relation between the infiltration by Langerhans cells and the prognosis using S-100 (P-value=0.007) and using CD1a (P-value=0.003). However, no relation was shown with other prognostic factors. **Conclusion:** The study showed that better prognosis correlated with higher degree of Langerhans cells infiltration which indicates an important role in expecting prognosis during the treatment of larynx malignant tumers.

KEYWORDS: Larynx cancer, Langerhans cells, S-100, CD1a.

INTRODUCTION

Due to its fast development comparing to other tumers, laryngeal cancer is considered a major challenge, beside other factors like the important anatomical placement of the larynx which worsen the prognosis.[1,2,3] That makes it important to gain deeper understanding of the different factors that contribute to determining prognosis. Some factors' roles were approved, such as sex, protein intake, physical activity levels before diagnosis, TNM classification, and additional primary tumers. While other factors are still controversial such as age, haemoglobin levels on diagnosis, serum selenium, and a number of biomarkers.[4,5,6,7,8,9]

Langerhance cells were first described by Paul Langerhans in 1868 as dendretic cells in epidermis. They are Antigen-presenting cells originating from bone marrow, which play a key role is the defensive mechanismn by picking up antigens encountered in the squamous epithelia and migrating subsequently to the draining lymph nodes. Upon arrival in the paracortex of lymph nodes, they transform into interdigitating cells and they present antigen to naive T lymphocytes.[10,11] Therefore these cells may be involved in immunosurveillance against neoantigens associated with malignant transformation by transporting antigens to local lymph and presenting them to specific T cells. Furthermore, many studies indicate that Langerhans Cells have a role in the immunosurveillance against a number of different tumors, such as nasopharyngeal carcinoma, gastric carcinoma, or papillary thyroid carcinoma. The studies also showed A relationship between Langerhans Cells and prognosis in many tumors, but only a few studied this relationship with larynx cancer.

PATIENTS AND METHODS

This is a retrospective cohort study of a group of patients attending the department of ENT in Tishreen University Hospital in Latakia, Syria between 2017 and 2020. The inclusion criteria were patients who were suspected to have larynx cancer, males or females, their biopsies showed malignancy, and they had they undergone Laryngectomy. The exclusion criteria were patients with previous story of chemotherapy, or previous radiotherapy, patients who were receiving immunodepressors, patients whose data were missing. The number of studies patients was 21, forms were made for patients containing information such as: name, age, sex, medical and surgical history, habits and instincts, occupational exposur. Langerhance cells infiltration was studied twice using s-100 and using cd1a, in both patient were classified into 4 categories: when no infiltration was seen, the degree given was 0, little infiltration was given the degree 1, intermediate infiltration was given the degree 2, important infiltration was given the degree 3. The N classification and langerhance infiltration degree were added to the forms.

Ethical consideration: This study was performed in accordance with the Declaration of Helsinki.

Statistical Analysis
Statistical analysis was performed by using IBM SPSS version 20. Basic Descriptive statistics included means, standard deviations (SD), Frequency and percentages. To examine the relationships and comparisons between the groups, chi-square test was used. Independent t student test was used to compare independent groups. All the tests were considered significant at a 5% type I error rate($p<0.05$), β:20%, and power of the study:80%.

RESULTS

The study included a group of 21 patients who had larynx cancer and had laryngectomy. The baseline characteristics of patients were as shown in Table (1). Age ranged from 41-76 years, with a mean age of 58.6 years. Males represented 76.2% and females 23.8% of the patients. 16 patients were smokers (76.2%) and 4 were alcoholic (19%). Spreading to the lymph nodes was seen in 8 patients (38.1%). Two patients were diabetic (9.5%)

Table 1: Demographic characteristics of the study population.

Variable	Result
Age(years)	
40-50	23.8%
50-60	28.6%
60<	47.6%
Gender	
Male	16(76.2%)
Female	5(23.8%)
Habits	
Smoking	16(76.2%)
Alcohol	4(19%)
spread to the lymph nodes	8(38.1%)
Diabetes	2(9.5%)

Patients' pathology samples were studied using S-100 then using CD1a, the samples were classified into four grades for each straining; when no infiltration was seen the sample was given grade 0, grade 1 for little infiltration, grade 2 for intermediate infiltration, and grade 3 for important infiltration.

3-years survival rate was 23.8%, when studying with S-100 no infiltration with Langerhans cells was seen in 2 patients(9.5%-degree 0), little infiltration(degree 1) was seen in 8 patients(38.1%), intermediate infiltration (degree 1) was seen in 8 patients(38.1%), important infiltration was seen in 3 patients(14.3%). When studying with CD1a no infiltration(degree 0) was seen in 2 patients(9.5%), little infiltration(degree 1) was seen in 11 patients(52.4%), intermediate infiltration (degree 2) was seen in 6 patients(28.6%), important infiltration (degree 3) was seen in 2 patients(9.5%).

The Table 2: Shows the infiltration of Langerhans cells using S-100 and CD1a.

Degree	Degree 0	Degree 1	Degree 2	Degree 3
S-100	2(9.5%)	8(38.1%)	8(38.1%)	3(14.3%)
CD1a	2(9.5%)	11(52.4%)	6(28.6%)	2(9.5%)

The Table 3: Shows the relation between 3-years prognosis and Langerhans cells infiltration on S-100 immunostraining.

Cells infiltration on S-100		Degree 0	Degree 1	Degree 2	Degree 3	P-value
3-years prognosis	survival	0	0	2	3	0.007
	Death	2	8	6	0	

The Table 4: Shows the relation between 3-years prognosis and Langerhans cells infiltration on CD1a immunostraining.

Cells infiltration on CD1a		Degree 0	Degree 1	Degree 2	Degree 3	P-value
3-years prognosis	survival	0	0	3	2	0.003
	Death	2	11	3	0	

The Table 5 Shows the relationship between different variables and Langerhans cells infiltration when studied using S-100.

Cells infiltration on S-100		Degree 0	Degree 1	Degree 2	Degree 3	P-Value
Sex	Male	2	6	5	3	0.88
	Female	0	2	3	0	
Smoking	Yes	2	6	2	2	1
	No	0	2	6	1	
Alcohol	Yes	1	1	1	1	0.3
	No	1	7	7	2	
Diabetes	Yes	1	0	1	0	0.2
	No	1	8	7	3	
Age	40-50	0	2	2	1	0.96
	50-60	1	2	1	1	
	Above60	1	4	5	1	
Tumer spreading to Nodes	Yes	1	3	4	0	0.53
	No	1	5	4	3	

The Table 6: Shows the relationship between different variables and Langerhans cells infiltration when studied using CD1a.

Cells infiltration on CD1a		Degree 0	Degree1	Degree 2	Degree 3	P-value
Sex	Male	2	8	4	2	1
	Female	0	3	2	0	
Smoking	Yes	2	8	4	2	1
	no	0	3	2	0	
Alcohol	Yes	1	2	0	1	0.1
	no	1	9	6	1	
Diabetes	Yes	1	1	0	0	0.4
	no	1	10	6	2	
Age	40-50	0	1	3	1	0.12
	50-60	1	3	0	1	
	Above60	1	7	3	0	
Tumer spreading to nodes	Yes	1	6	1	0	0.43
	No	1	5	5	2	

The table 3 shows stastically important relation between the infiltration by langerhans cells as seen using S-100 and 3-years survival (P-value=0.007), and the higher grade of infiltration with Langerhans cells associated with better prognosis.

The table 4 shows stastically important relation between the infiltration by langerhans cells as seen using CD1a and 3-years survival (P-value=0.003), and the higher grade of infiltration with Langerhans cells associated with better prognosis.

Tables 5 and 6 showed no statically important relation between Langerhans cells infiltration using S-100 with any of the variables studied (sex, smoking, alcohol, diabetes, age, or spreading to nodes) (P-values were: 0.88, 1, 0.3, 0.2, 0.96, 0.53 respectively), and no statically important relation either between Langerhans cells infiltration using CD1a with any of the variables studied(sex, smoking, alcohol, diabetes, age, or spreading to nodes) (P-values were: 1, 1, 0.1, 0.4, 0.12, 0.43 respectively).

DISCUSSION
Larynx cancer is on of the most challenging tumers, due to its relatively rapid development, the special anatomical placement of the larynx that worsen prognosis, the functional importance of the larynx and the high costs of treatment, and the difficulty determining best treatment approaches that requires a significant experience.[1,2,3] All these facts make it crucial to deeply understand this tumer, and one big question to answer is about factors that help determining prognosis. Many prognostic factors were studied and approved to have effect on prognosis like sex, nutritional state, physical activity, TNM staging. etc, while some prognostic factors are still being studied like

haemoglobin levels on diagnosis, serum selenium levels, and biomarkers.[4,5,6,7,8]

Our study revealed a statically important relation between Langerhans cells infiltration in tumer stroma and prognosis, that results were seen when studied using two different immunostrainings seperately, S-100 (P-value=0.007), and CD1a (P-value=0.003). better prognosis was noticed associated with higher grade of Langerhans cells infiltration in tumer stroma, this may be explained by the immunofunctions of Langerhans cells as antigen presenting cells, beside their ability of migration to places of need, they can have role in killing tumer cells or in recognizing tumer antigens making immunoresponse to that tumer more effective later therefore decreasing recurrence rates and improve prognosis.

Kindt, Nadege et al studied 25 tumor-free peritumoral epithelium, 64 low-grade dysplasia, 54 high-grade dysplasia and 125 carcinoma samples, and demonstrated that high Langerhans number is associated with longer recurrence-free survival in both intra-tumoral and stromal compartments and longer overall survival in stromal compartment, in a study puplished in November, 2016.[12]

Karakök, Metin, et al conducted a study in Gaziantep University in 2003, on 45 patients but didn't find a statically important relation between Langerhans cells infiltration and prognosis (P-value=0.166>0.05).[13]

Gallo, Oreste, et al showed in a study in 1991 in University of Florence on 88 samples using S-100 protein, their study showed no correlation between Langerhans cells number and clinical stage or invading lymph nodes. However, the study showed significant correlation between the number of these cells and prognosis, intermediate and high density of Langerhans cells infiltration correlated with 5-years survival rates of 61% and 62% respectively, while low density correlated with 0% 5-years survival rate.[14]

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