Role of abnormal Langerhans cells in oral epithelial dysplasia and oral squamous cell carcinoma: A pilot study

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Abstract

Background: The oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC), although initiated by tobacco carcinogens, their progression is due to inability of Langerhans cells (LCs) to detect these abnormal cells and promote lymphocytes to destroy these cells. We assessed and quantified the tumor associated LCs and inflammation in OED and OSCC to understand their role. **Materials and Methods:** Fifty-five microscopic sections were assessed (27 OED and 28 OSCC). The LCs were detected using S-100 immunohistochemical marker. The number of tumor associated LCs were counted. The presence of abnormal appearing large cells and its relation to histopathologic grade and inflammation was assessed. **Results:** Significant increase in the LC count was observed in OSCC when compared to dysplasia. Large, abnormal appearing cells were observed in dysplasia and carcinomas however, these were more pronounced in moderate dysplasia and poorly-differentiated carcinomas. The presence of these abnormal appearing cells was associated with decrease in lymphocytic infiltrate. **Conclusion:** The present study indicates more LC are recruited into the carcinoma. These accumulated nonfunctional LC in the tumor tissue are indicative of aggressive tumor with potential malignant transformation.

Key words: Inflammation, Langerhans cell, oral epithelial dysplasia, oral squamous cell carcinoma, S-100

INTRODUCTION

In India, oral squamous cell carcinoma (OSCC) is the most common cancer among men and fourth most common cancer among women and accounts for one-third of the world cancer burden.[1,2] The major etiological factors are the use of the tobacco either in smoking and/or chewing forms and alcohol. The clinical changes (leukoplakia, erythroplakia or oral submucous fibrosis) are associated with the spectrum of histopathological changes starting from epithelial hyperplasia to different grades of dysplasia eventually progressing to cancer.[3,4]

Langerhans cells (LCs) are dendritic antigen presenting cells,[5,6] which originate from the bone marrow and migrate into the stratified squamous epithelium of the skin and mucosa of the upper aerodigestive tract.[7] The function of LCs is to recognize antigen, process it and present it to T cells. They intercept and bind new antigens detected in the squamous epithelium. They are responsible for initial stimulation of naïve T lymphocytes and secondary immune response by stimulating memory T cells.[8,9] In the oral mucosa other than LCs, melanocytes and Schwann cells can express positivity for S-100 antibody. However, these cells can be easily distinguished from other S-100 positive

cells based on intraepithelial distribution, association with inflammatory cells and peculiar dendritic morphology.[10] We compared the distribution pattern and types of LCs in oral epithelial hyperplasia with leukoplakia, different grades of dysplasia and in carcinoma. We also analyzed the relationship of LC with the density lymphocytic infiltrations in the tumor-associated connective tissue.

MATERIALS AND METHODS

Buffered formalin fixed, paraffin wax embedded archival tissue blocks obtained from Department of Oral Pathology and Microbiology at our institution were used in this study. All the cases included in the category of dysplasia of hyperplasia were diagnosed clinically as leukoplakia. Histologically they were classified as epithelial hyperplasia, mild dysplasia, moderate dysplasia, and severe dysplasia. All the OSCCs were graded as well-, moderately- and poorly-differentiated carcinoma. Complete data on the type and duration of the habits was not available for all the patients, hence this criteria was not included in the study. Data collected included gender and age of the patients. The histological diagnosis was confirmed by review of the original Hematoxylin and Eosin slides by an oral pathologist. Ethics approval was obtained from the Institutional Human Ethics Committee.

Immunohistochemistry

Four μm sections of formalin-fixed, paraffin-embedded tissues were mounted on 3-aminopropyltriethoxysilane coated slides and incubated at 27°C for 24 h. Sections were then deparaffinized using xylene, followed by hydration using descending grades of alcohol. Antigen retrieval was performed in citrate buffer using pressure cooker. Endogenous peroxidize activity blocking was done using 3% hydrogen peroxide in distilled water for 10 min. After a tris buffer wash, nonspecific antigen blocking was done by incubation with power block solution. Sections were incubated in primary antibody at room temperature in 100% moisture for 60 min. The primary antibody was prediluted, purified bovine S-100 protein (Biogenix, AM058-5M) raised against mouse. Following the primary antibody further staining was performed using SuperSensitive™ polymer-HRP-immunohistochemistry (IHC) Detection System with DAB as a chromogen (BioGenex Laboratories, SanRamon, CA, USA) according to the manufacturer's protocol. Known control section (normal oral mucosal tissue) was included for each batch of staining to confirm the presence of appropriate immunostaining activity. Negative controls were included to assess the nonspecific staining. All slides were counter stained with hematoxylin and after dehydration were mounted with DPX.

Visualization and analysis

Langerhans cells were identified using the following criteria:
a. Brownish colored S-100 positive cells.
b. Elongated, pyramidal, round to ovoid brown stained cell with clearly visible cell body.
c. Presence of at least one dendritic process seen radiating from the cell surface.

All the slides were analyzed and counted independently by three senior oral pathologists using ×40 magnification of binocular light microscope. All the oral pathologists were blinded with respect to the clinical and histopathological details. During counting, the photographs of the counting field were obtained using photomicrograph. Any discrepancy of more than 5 LC cells slides were recounted and consensual agreement was obtained after recounting using the photographs. Mean value of all the three counts were entered in the data sheet. The number of LC were counted as total number of cells per high power field. The total LC associated with the tumor and in the peritumoral connective tissue were also counted. We classified the LC as:
a. Normal-When the all the cells showed normal appearance that is, pyramidal or small round to oval shaped cells with dendrites.
b. Few large cells-When at least 25% of the cells in 10 high power fields show large, abnormal appearing, irregular round to ovoid shape.
c. Predominantly large cells-When more than 25% of the cells in 10 high power fields show abnormally large cells.

The lymphocytic infiltration in the connective tissue was graded as mild, moderate and dense. Data were collated into a spreadsheet and analyzed using SPSS version 10 (IBM corporation, Somers, NY, USA).

RESULTS

A total of 55 cases were included in the study. Of these, 28 were OSCC and 27 were oral leukoplakia. The mean age of the OSCC group was 51 years (range: 31-70 years, standard deviation = 10.72). The mean age of oral epithelial dysplasia (OED) group was 52.85 years (range: 24-85 years, standard deviation = 16.46). All the patients were males, except for 2 patients in carcinoma and 3 in dysplasia being females.

A total number of S-100 positive LC were compared at different sites. In both the dysplasia and SCC of the tongue, increase in LC was observed. There was definite increase in the total number of LC in OSCC compared to OED at all the sites [Table 1].

The total number of LC was counted in different grades of OED and OSCC [Table 2]. The total LC in different grades of dysplasia and OSCC was found to be statistically significant ($P < 0.05$, one-way ANOVA) [Figure 1].

A number of cases with different types of LC were compared in well-, moderate- and poorly-differentiated OSCC as well as different grades of dysplasia. Although, hyperplasia was into associated with any abnormal LC, abnormal cells (category b and c) were observed in both dysplasia and OSCC [Figures 2-7]. Interestingly moderate dysplasia cases and poorly-differentiated carcinomas showed more abnormal LC [Figure 8]. A number of S-100 positive LC were compared in the presence of mild, moderate and dense chronic inflammatory cell infiltrate in both dysplasia and SCC [Table 3]. There was increase in the number of LC in OSCC associated with mild inflammation when compared to moderate inflammation. In OED, LC were higher in mild and dense inflammation when compared to moderate inflammation. Types of LC were associated with different grades of inflammation in OED and OSCC [Table 4]. In OSCC, the mild inflammation was associated with the larger LC. This may indicate the failure of LC to perform their function and hence accumulate in the tumor.

DISCUSSION

The LC are intraepithelial dendritic cells (DCs) which process and present antigens to the immune cells, playing important role in tumor immunity.[9] The pyramidal shaped cells with long dendritic process located in suprabasal layer are Type I LC. The spherical cells with shorter DCs located in the basal layer are Type II LC.[11] Analyzing LC associated with tumor component is useful in evaluating the immunologic status of the patient.[10] Studies done on LC counts in OSCC and dysplasia have shown significant decrease of these DCs in OSCC compared to dysplasia.[12,13] The reduction was conspicuous in higher-grade tumors. Contrary to these previous reports we observed statistically significant increase in the number of LC in OSCC when compared to OED. Although, increase in LC in the peritumoral connective tissue is previously reported,[14] it is suggested that the greater influx of LC in the invasive tumors reflects increased tumor bulk resulting in increased production of the putative chemotactic factors. Chemokine MIP-3/CCL20 produced by tumor cells are selectively chemotactic to LC.[15] Interleukin-10, transforming growth factor-alpha, vascular endothelial growth factor could

Table 1: Mean LCs in OSCC and dysplasia at different sites

S-100	Site	Number of patients	Mean LC
OSCC	Buccal mucosa	7	26.28±4.00
	Tongue	1	37.2963±14.16
	Lateral border of the tongue	9	
	Floor of the mouth	1	—
	Gingiva and alveolar ridge	7	24.5238±14.98
	Palate	3	26.0000±14.19
Dysplasia	Buccal mucosa	10	15.9667±9.10
	Dorsum tongue	2	35.8333±15.12
	Lateral border of the tongue	5	20.2667±14.64
	Floor of the mouth	0	—
	Gingiva and alveolar ridge	9	15.0000±6.85
	Palate	1	—

OSCC: Oral squamous cell carcinoma, LC: Langerhans cells

Table 2: Mean of total number of LCs in OSCC and different grades of dysplasia

S-100	Mean	Minimum	Maximum	SD
Well-differentiated OSCC	31.77	20.67	52.67	9.05
Moderately-differentiated OSCC	29.09	13.67	66.67	14.51
Poorly-differentiated OSCC	27.95	0.00	41.67	15.18
Hyperplasia	9.50	0.00	14.67	5.07
Mild dysplasia	16.76	7.67	22.00	5.73
Moderate dysplasia	25.30	10.00	56.00	16.13
Severe dysplasia	16.53	4.33	25.67	7.72

OSCC: Oral squamous cell carcinoma, LC: Langerhans cells, SD: Standard deviation

Table 3: Total number of S-100 positive LCs in OSCC and OED associated with different grades lymphocytic infiltrate

Inflammation	Number of cases	Mean S-100	Minimum	Maximum	SD
OSCC					
Mild	11	29.5758	0.00	66.67	17.47
Moderate	12	28.1667	22.00	38.67	5.20
Dense	5	34.0000	13.67	52.67	14.36
OED					
Mild	7	16.6667	4.33	25.67	7.247
Moderate	10	22.0333	14.77189	7.67	56.00
Dense	10	14.7667	0.00	41.00	10.64

OSCC: Oral squamous cell carcinoma, LC: Langerhans cells, OED: Oral epithelial dysplasia, SD: Standard deviation

also regulate the recruitment and migration in the tumor microenvironment.[16]

Pronounced decrease of LC in poorly-differentiated OSCC is previously reported.[17,18] The opposite was observed in

Table 4: Number of cases showing different types of LCs were compared with different grades of inflammation in OED and OSCC

Inflammation	Type of LC				Total
	No LC	Normal	Few LC	Predominant LC	
OSCC					
Mild	1	4	1	5	11
Moderate	0	6	3	3	12
Dense	0	2	1	2	5
Dysplasia					
Mild	0	4	0	3	7
Moderate	0	6	1	3	10
Dense	1	5	1	3	10

OSCC: Oral squamous cell carcinoma, LC: Langerhans cells, OED: Oral epithelial dysplasia

moderately- and well-differentiated tumors, which was associated with possible immune suppression induced by anaplastic tumor cells. Our observations are consistent with this report. However, lack of correlation between grading and ploidy status or LC count is also reported.[19] The number of LC infiltrating the tumor is a significant prognostic factor in OSCC patients. The increase in number of LC is regarded as a better prognostic factor.[20]

Increase in the LC density was seen at the lateral border of tongue and lip in subjects with history of smoking.[21] The tobacco smoke may cause increase in the local LC population helping to maintain normal protective function of oral mucosa. This is possibly due to increased rate of cell division of resident LC or influx of precursors from the circulation. Indeed smokeless tobacco usage reduced the number of LC due to inhibitory effect from the absorption of the tobacco contents.[22] In our cases, all the patients had the habit of tobacco chewing and some of them had
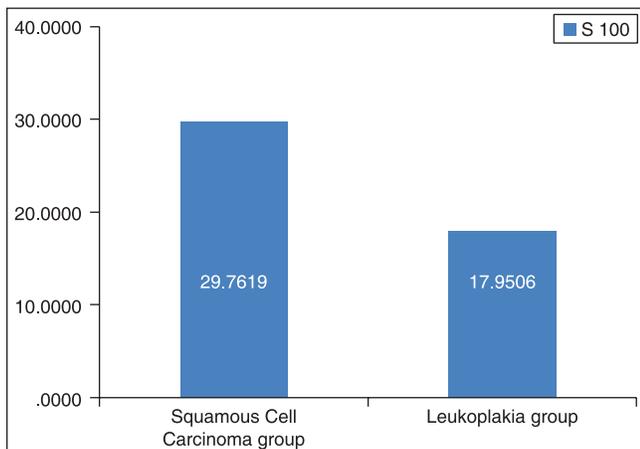
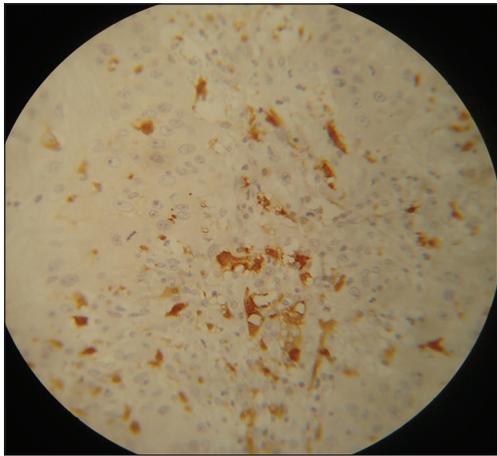**Figure 1:** The mean total number of S-100 positive Langerhans cells in squamous cell carcinoma and dysplasia

Figure 2: Normal appearing Langerhans cells in hyperplasia (Immunohistochemistry, ×40)
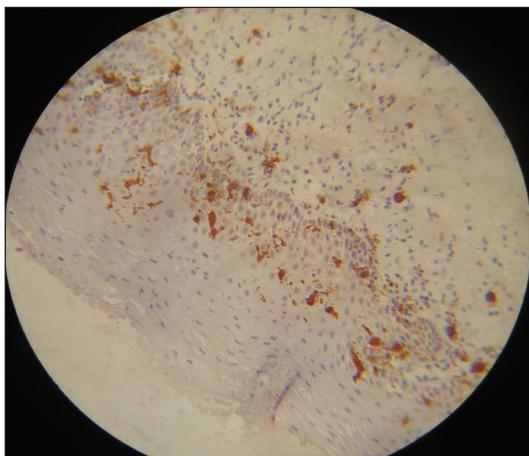
Figure 3: Numerous large abnormal appearing Langerhans cells (LCs) interspersed with normal appearing LCs in moderate dysplasia, connective tissue shows minimum inflammation (Immunohistochemistry, ×40)
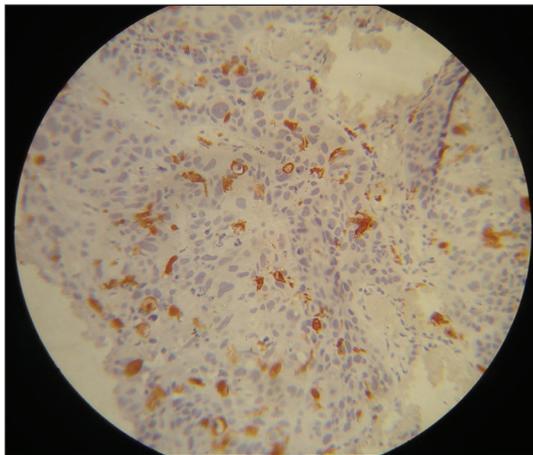

Figure 4: Well-differentiated squamous carcinoma with abnormal appearing Langerhans cell (Immunohistochemistry ×40)

Figure 5: Moderately-differentiated squamous cell carcinoma showing large abnormal appearing Langerhans cell (Immunohistochemistry, ×40)

Figure 6: Moderately-differentiated squamous cell carcinoma showing normal appearing Langerhans cell (Immunohistochemistry, ×40)

Figure 7: Poorly-differentiated squamous cell carcinoma with abnormal Langerhans cell and minimum inflammation (Immunohistochemistry, ×40)
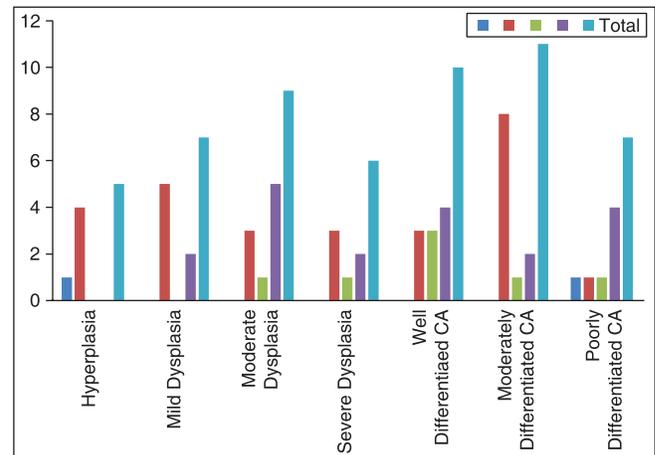
Figure 8: Number of cases with different types of S-100 positive Langerhans cells in dysplasia and oral squamous cell carcinoma

smoking and chewing habits. However, the total duration and type of tobacco usage could not be obtained from the case records.

After the antigen capture, LC migrates to regional lymph nodes. During this migration, they undergo morphologic and ultrastructural modifications[23,24] as a result of the maturation process. Numerous peculiar S-100 positive, round-shaped cells scattered in the connective tissue as a result of phenotypic changes, during their migration toward regional lymph nodes is reported[13,25,26] wherein these cells may participate in antitumor immunity.[27] In contrast presence of immature CD207/Langerin[+] in the primary OSCC and mature DCs were rare.[28] In our study, we found higher number of larger LC in moderate dysplasia and poorly-differentiated SCC. These altered LC may indicate accumulated immature cells. Due to reduced tumor immunity such lesions may behave aggressively.[28]

Antitumor response is initiated when immature LC come in direct contact with tumor cells. Defective host antitumor immune response causes immune evasion of the tumor cells often with invasion of immature immune cells.[29-32] Nevertheless an interesting question, which needs to be answered is, the role of LC in tumor immunity. The progression of tumor is unchecked even after increase in the number of infiltrating LC.[33] Low immunogenicity of the tumor cells may be the reason for lack of antigen recognition resulting in tumor cell immune evasion. Further, the functional capability of LC may be compromised within the tumor stroma[33] and the local environment of the dysplastic epithelium may further modulate the LC distribution.[34-37]

Increase in abnormal LCs in OED and OSCC associated with the mild inflammation in our study may be due to immune modulation by the locally secreted cytokines or due to immune escape. The reduction in the tumor immune response is reflected as lymphocytic infiltrate.

Thus, the local immune modulation and absence of fully functional LC result in reduced immune response resulting in aggressive tumor. We conclude that an important observation of abnormal LC associated with the OEDs and OSCC and their association with reduced lymphocytic infiltrate reflects poor tumor immunity and aggressive tumor. Further studies are necessary to investigate the possibility of simple S-100 IHC as a surrogate marker for the surgeons to decide the aggressive treatment options in dysplasia and OSCC.

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