

## Role of Langerhans Cells in Oral Epithelial Dysplasia and Squamous Cell Carcinoma

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### Original Research Article

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**Abstract:** Langerhans cells are dendritic, antigen-presenting cells which reside within the stratified squamous epithelium of skin and various mucosae, where they function as the outermost arm of the immune system. Langerhans cells play an important role in inflammation and immune responses, including anti-tumour activity. Thus this study is aimed to identify the role of Langerhans cell in oral dysplastic epithelium and oral squamous cell carcinoma. Quantitative analysis of Langerhans cells were done in normal oral mucosa (n=10), oral dysplastic epithelium (n=20) and oral squamous cell carcinoma (n=20) with immunohistochemical analysis using monoclonal antibody against CD1a. Counting was done in ten non-overlapping consecutive high power fields by two independent observers. An increase in the number of Langerhans cells were observed from normal mucosa to oral dysplastic epithelium and oral squamous cell carcinoma. There was no statistically significant increase observed from oral dysplastic epithelium to oral squamous cell carcinoma. The immune system responds to the dysplastic and invading epithelium in oral squamous cell carcinoma by recruiting Langerhans cells. Langerhans cells present altered antigen to naïve T cells, thus initiating an immune response. However, tumour microenvironment has a critical role in modulating the recruitment and function of Langerhans cells in oral squamous cell carcinoma

**Keywords:** CD1a, Dendritic cells, Langerhans cells, oral dysplastic epithelium, oral squamous cell carcinoma.

### INTRODUCTION

Oral dysplastic epithelium is considered a potentially malignant disorder with a very high rate of malignant transformation of approximately 16% in severe, 3%-5% in moderate and less than 5% in mild dysplasia [1]. 40% of all the malignancies reported in India falls under head and neck cancers of which oral cancer accounts for 30%. 90% of all oral cavity cancer is histologically proven to be squamous cell carcinoma [2]. Head and neck squamous cell carcinomas (HNSCCs) remain a significant cause of morbidity worldwide despite the advances in the treatment modality. Origin of head and neck cancer is linked to environmental carcinogens (tobacco, alcohol etc.) whereas tumor progression could be linked to a failure of the immune system to fight against cancer [3]. The concept of immune surveillance of cancer proposed by Burnet and Thomas in 1950 suggests that the immune system of the body can recognize tumour cells as abnormal cells and destroy them [4].

First visualized in 1868, Langerhans cells has since then witnessed numerous speculations regarding

its function. They are now proved to be a subset of Dendritic cells and functions as immune-surveillant units in the mucosal linings [5]. Among the various surface markers they express, CD1a is one of the most specific marker of Langerhans cell [6].

Thus this study involved quantitative analysis of Langerhans cells in oral dysplastic epithelium and oral squamous cell carcinoma and its role in malignant transformation

### MATERIALS AND METHODS

#### Patient samples

This laboratory based retrospective study involved the use of buffered formalin fixed, paraffin embedded tissues of previously histopathologically diagnosed cases of oral epithelial dysplasia and oral squamous cell carcinoma, retrieved from the archives of Department of Oral & Maxillofacial Pathology of our college.

A total of 50 tissue sections were selected which included 20 tissue sections each of oral epithelial

dysplasia and oral squamous cell carcinoma and 10 sections of normal oral mucosa Haematoxylin and eosin stained sections were evaluated for the presence of the lesion and also for the adequacy of the connective tissue depth. Tissue sections utilized for the study included only those of excisional biopsy specimen of oral squamous cell carcinoma which contained sufficient epithelial component as well as stroma for evaluation.

Clinically normal oral mucosa tissue was collected from posterior buccal sulcus/retromolar area during disimpaction procedures from the Department of Oral and Maxillofacial Surgery of our institution.

Ethical clearance for the use of these paraffinised tissue blocks were obtained from the Institute.

#### **Immunohistochemistry**

The procedure followed in the immunohistochemical staining is as per the instructions given by Biogenex Ltd. The primary antibody used was Antihuman CD1a (Q BEND/10, Biogenex), 1:25 dilution, taken from Biogenex life sciences limited (CA, USA).

A super sensitive polymer – HRP detection system, a biotin free detection system supplied by biogenex life sciences limited (CA, USA) was used as the visualization kit which carried the chromogen Diaminobenzidine (DAB).

The tissue sections of 4 $\mu$  were prepared from the selected paraffin embedded tissue blocks and incubated overnight at 37 °C. Antigen retrieval following deparaffinisation was done by heat retrieval method using citrate buffer solution. The slides were counterstained with Harris haematoxylin and later mounted using DPX.

#### **IHC scoring**

The Langerhans cell counting was performed with a binocular, light microscope under high power magnification (40X). Ten successive high power fields were selected in dysplastic epithelium. In oral squamous cell carcinoma, ten fields selected included the invading epithelial islands. The total count was divided by the ten to get the average Langerhans cells in each field. In each slide cells were counted in step ladder pattern to avoid recounting of the same areas irrespective of number of fields. Two independent observers quantified each slide to reduce the inter-observer error.

#### **Statistical analysis**

t test and Mann-Whitney U test were done to test the consistency between the two observers. To compare the significance between the three groups, a non-parametric testing method using Kruskal-Wallis and Mann-Whitney U tests were carried out.

#### **RESULTS AND DISCUSSION**

Among the cases of oral dysplastic epithelium, 85% of cases were males and 15% were females. The age ranged between 29- 74 years. The habit history of these individuals varied from chewing and smoking tobacco in various forms to consumption of alcohol. Among these, chewing tobacco was most commonly practiced habit recorded (up to 50 years of tobacco chewing). These cases presented clinically as mixed red and white lesions, white keratotic patches and as ulcers and the most frequent site involved was the buccal mucosa especially on the right side of the oral cavity (45%). This was followed by involvement of left buccal mucosa (25%), lateral border of the tongue (15%), lip (10%) and the palate (5%). The clinical diagnosis of these cases was frequently speckled leukoplakia or homogenous leukoplakia.

Of the twenty cases of oral squamous cell carcinoma, 85% cases were male patients and the remaining 15% were females. The age of these patients ranged from 27-72 years. In all except four patients, habit history was noted with tobacco chewing as the most commonly practiced. Clinically, most of the patients presented with an ulceroproliferative lesion on the right buccal mucosa & maxillary alveolar ridge involving the gingivobuccal sulcus (25% each), lower lip and lateral border of tongue (20% each), followed by 10 % involvement of left buccal mucosa. At the time of presentation, ten patients were noted to have palpable lymphnodes, eight of them with palpable level I lymph nodes, and two of the remaining cases with palpable level I and II lymph nodes. Histopathologically, only three cases were found to be positive for metastatic deposits.

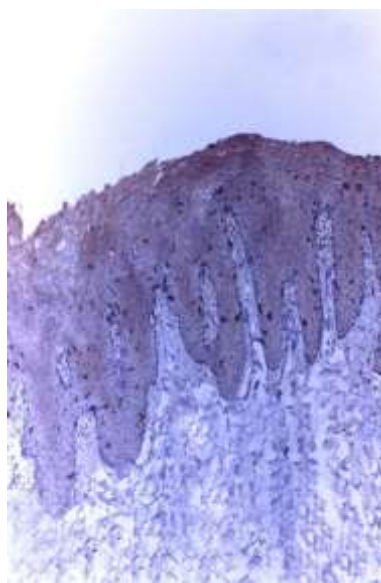
Under light microscope, the CD1a-positive LCs exhibited a membrano-cytoplasmic staining of uniform intensity of brown colour. The morphology of the LC varied from round to oval with dendritic cytoplasmic processes extending into adjacent epithelial cells. Only cells with a definable cell body and nucleus and at least one dendritic process were considered LC. t test and Mann-Whitney U test were carried out to test the consistency between the two observers. There was no statistically significant difference in values of Langerhans cells between the two observers in any of the three groups (Table-1).

**Table-1: Pair wise comparison of three study groups between the two observers for Langerhans cells density values by Mann-Whitney U test:**

Groups	n	p-value
N1	10	
N2	10	<b>0.959</b>
OED1	20	
OED2	20	<b>0.947</b>
OSCC1	20	
OSCC2	20	<b>0.968</b>

Langerhans cells were localized in the basal layer and the spinous layer in normal oral mucosa and were evenly distributed in the epithelium (Figure-1). A

reactive inflammatory response was observed in few cases as they were obtained from flaps elevated for disimpaction.



**Fig-1: Normal oral mucosa shows Langerhans cells in basal and spinous layer of epithelium. (IHC, DAB chromogen, CD1a monoclonal antibody, Original magnification x10)**

A statistically significant difference was observed in values of Langerhans cells (LC) between normal oral mucosa (N) and oral epithelial dysplasia

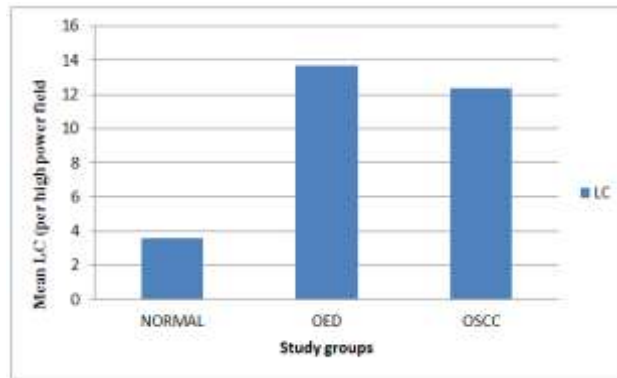
(OED) study groups and between normal oral epithelium and OSCC study groups (Table-2 & 3).

**Table-2: Pair wise comparison of Normal and OED study groups for LC values by Mann-Whitney U test**

Groups	n	Mean rank	Sum of ranks	p-value
Normal	10	7.80	78.0	<b>0.000</b>
OED	20	19.35	387.0	

**Table-3: Pair wise comparison of Normal and OSCC study groups for LC values by Mann-Whitney U test**

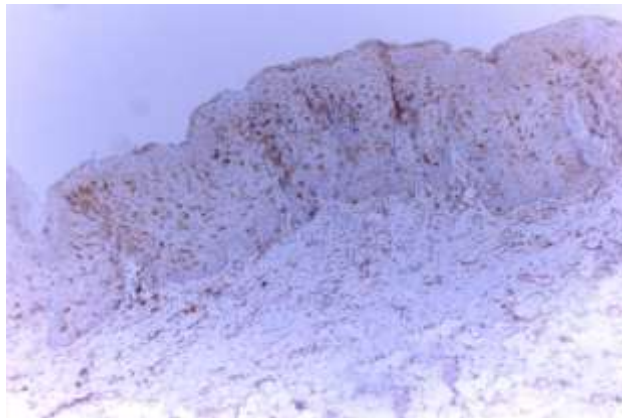
Groups	n	Mean rank	Sum of ranks	p-value
Normal	10	6.75	67.50	<b>0.000</b>
OSCC	20	19.88	397.50	



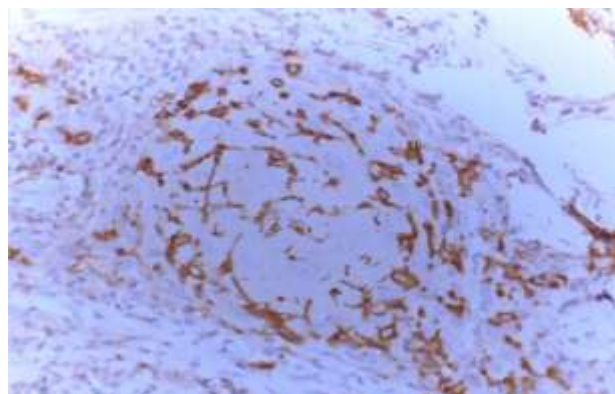
**Graph-1: A significant recruitment of LCs in OED and OSCC as compared to control was observed.**

In oral epithelial dysplastic lesions and oral squamous cell carcinoma, LC were distributed in the basal, spinous and corneum layer (Figure-2).

In squamous cell carcinoma, LC was quantified at the invasive front (photomicrograph 3). In case of OED and OSCC, there was a huge variation in the quantification of Langerhans cells within the group (1.6-38.6).



**Fig-2: Oral epithelial dysplasia shows dense distribution of LCs in the epithelium. (IHC, DAB chromogen, CD1a monoclonal antibody. Original magnification x10)**



**Fig-3: Oral squamous cell carcinoma shows dense distribution of Langerhans cells in epithelial island at the invasive front. (IHC, DAB chromogen, CD1a monoclonal antibody. Original magnification x40)**

Statistically significant difference was not observed in values of Langerhans cells (LC) between

oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) study groups (Table-4).

**Table-4: Pair wise comparison of OED and OSCC study groups for LC values by Mann-Whitney U test**

Groups	n	Mean rank	Sum of ranks	p-value
OED	20	20.65	413.00	<b>0.968</b>
OSCC	20	20.35	407.00	

During the initiation, promotion, and progression of multi-step carcinogenesis, changes in host immunological factors have been observed. Inadequate presentation of tumour antigens by host LC is one potential mechanism that allows tumor progression. A rapidly growing body of literature suggests a pivotal role for LCs in various immune and inflammatory responses, including anti-tumour immunity. In tumor-bearing sites LCs uptake, process, and present tumour-associated antigens to naive or memory T-cells, which leads to generation of tumour-specific effector T-cells, capable of recognizing and eliminating tumour cells. Immune tolerance develops due to altered function of LCs, which subsequently allows proliferation of aberrant cells.

The findings of distribution pattern and density of LC in normal epithelium in our study were no different from the other previously reported studies [7, 8].

As the mucosa undergoes changes due to various environmental factors, the cells begin to show dysplastic features which are perceived by the immune system as 'antigens'. The immune system thus recruits more LCs to the site as a defense mechanism in immune-competent individuals. This explains the increase in LCs from the control group to dysplastic epithelium and oral squamous cell carcinoma. A further increase is contributed in response to the various other factors like secondary infection of the lesion and trauma. Various other studies have also confirmed this finding [7].

This also explains an increase in the LC in oral epithelial dysplasia and squamous cell carcinoma when compared to the normal mucosa in our study.

Variation in the quantification of Langerhans cells within OED has been observed in previous studies which included the different grades of dysplasia into consideration. This variation could be attributed to the decrease in LCs with increasing grades of dysplasia. This could be correlated clinically to the increased chances of severe dysplastic lesions transforming into malignant lesions due to defective immune response [9]

Other factors like tobacco smoking and chewing have been reported to decrease the number of LCs in mucosal potentially malignant lesions [10] and alterations in the number of LCs in tobacco-associated lesions is a result of changes caused by tobacco, and is one factor in the long-term pathogenesis of oral carcinoma [11]. It was proposed that LCs participate in the

pathogenesis of mucosal pathology that precedes oral cancer or depletion of LCs can decrease the oral immunity resulting in pathogens causing more damage [12].

Mechanisms that could have led to an increase in the LC infiltration of the dysplastic tissues are: (a) reduced migration to the lymph nodes; and/or (b) increased recruitment into the epithelium.

It has also been suggested that the factors released by the dysplastic/neoplastic cells inhibit the LC migration to lymph nodes leading to accumulation of LCs within the epithelial compartment. Furthermore various oral carcinogens are known to induce production of cytokines, like PGE2, IL-6, and TNF- $\alpha$ , by oral epithelial cells which are immuno-suppressive and known to inhibit LC differentiation, maturation and function. Thus, the local environment of the dysplastic epithelium may further modulate the LC distribution [13].

An increase in LCs count has been reported in various carcinomas in oral cavity as well as elsewhere in the body by numerous experiments conducted worldwide. LC count has also been correlated with the recurrence, survival rates and lymph node metastasis in the literature.<sup>[13]</sup>

In our study a statistically significant increase in the Langerhans cell count was not found from oral epithelial dysplasia to squamous cell carcinoma.

There are various hypothesis put forward to why an invasive lesion does not show an increase in the LC count. Homer et al. reported that serum vascular endothelial growth factor (VEGF) was raised in patients with head and neck tumors thus suggesting that tumour microenvironment has a negative influence on DC maturation [14]. In a study, Gabrilovich *et al.*, found that the production of VEGF by human cancer cells inhibited the functional maturation of DCs from CD34+ cells [15].

Several tumor-derived factors such as VEGF, IL-6, IL-10, and M-CSF have also been shown to be responsible for systemic and local DC defects. Furthermore, tumour metabolites such as lactic acid may also critically contribute to DC dysfunction and tumour immune escape.<sup>[15]</sup> Almand B et al. in his study suggested that tumour-derived factors such as IL-10 can induce dendritic cell apoptosis. Many studies have implicated IL-10 in the inefficient induction of tumour immunity [16].



To conclude, through this study, we have attempted to correlate the density of Langerhans cells in oral epithelial dysplasia and oral squamous cell carcinoma using monoclonal antibody against CD1a, and it has provided us with information regarding the immune response. The results indicate an immune activation in response to dysplastic and malignant cells in these lesions. However, the CD1a counts in ED & SCC do not show any statistical significance. Although the English literature has sufficient number of studies that questions the role of dendritic cells in various carcinomas, only few studies have correlated its role in oral epithelial dysplasia and oral squamous cell carcinoma. Targeted therapy enhancing the functions of LC in OED & OSCC might prove beneficial and improve prognosis.

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