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Non-dendritic Langerhans cells: A new entity in normal and malignant buccal mucosa

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SUMMARY

Langerhans cells (LCs) are the most effective antigen presenting cells against foreign bodies and carcinogens. Since the oral cavity is a portal of entry for these antigens, the aim of this study was to morphologically classify CD1a+ LCs, quantify them in the normal and malignant buccal mucosa, and evaluate their relation to the age of patients. Healthy buccal mucosal samples collected from 16 patients undergoing reconstructive operation, and malignant samples obtained from 15 patients undergoing radical oncological resection. were processed for immunohistochemistry four- to five-micron thick sections were stained with CD1a antibody (CD1a). At 40X magnification, CD1a+ LCs were morphologically classified and quantified manually for a 25mm length of basement membrane using Cellsens image analysing software and the data was analysed. Two categories of CD1a+ LCs were identified in the normal and malignant buccal mucosa a) typical dendritic LCs and b) non-dendritic LCs (a new entity). Non-dendritic LCs were of significantly higher number compared to the typical dendritic LCs in the normal tissues (p -0.001). In the malignant group, the non-dendritic CD1a+ LCs were significantly fewer in number (p-0.004), when compared to the normal group. Non-dendritic LCs were also significantly fewer (p-0.026) in patients over 60 years of age. This is the first report of non-dendritic Langerhans cells in normal buccal mucosa and malignant buccal mucosa using the CD1a marker. The significantly higher number of these cells in normal tissues and younger individuals supports their role as accessory antigen presenting cells.

Key words: Dendritic – Antigen presenting cells – Types of Langerhans cells – Accessory antigen presenting cells

INTRODUCTION

Langerhans cells (LCs) are professional antigen presenting cells with the ability to engulf and process both pathogens and foreign bodies (Steinman, 1991); (Steinman and Cohn, 1973). LCs have been previously classified into five types based on their dendritic processes and branching pattern (Figueroa and Caorsi, 1980). Cluster of differentiation (CD) 1a immunomarker is a specific and sensitive marker for LCs (Toews et al., 1980). This study was designed to describe the morphology and distribution of the CD1a+ LCs in the normal and malignant buccal mucosa.

MATERIALS AND METHODS

With the approval of the Institutional Review

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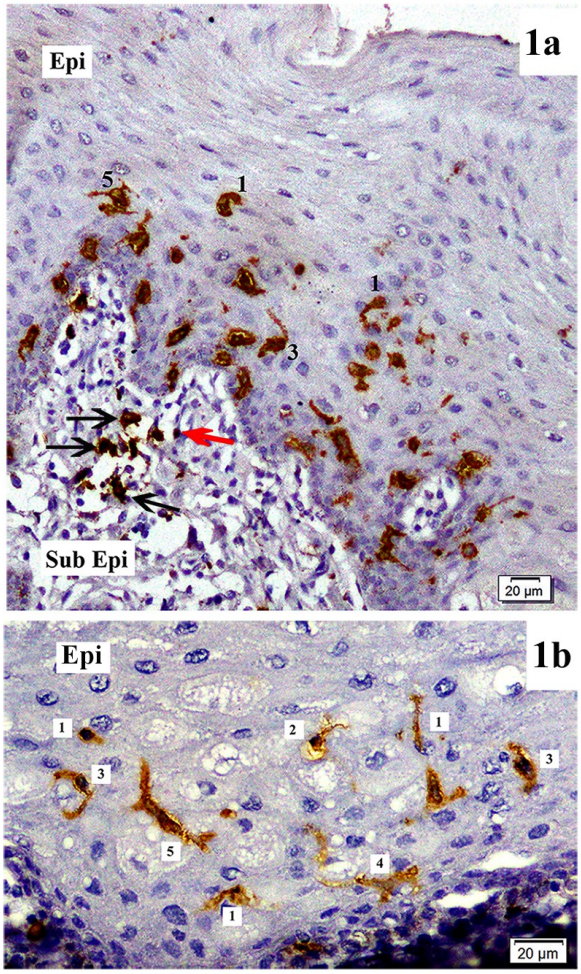


Fig 1. (a,b): Typical dendritic CD1a$^+$ LCs located in the stratified squamous epithelium (Epi) and the subepithelium (Sub Epi) of normal buccal mucosa.

1. Type I- LCs having a single process

2. Type II- LCs having a single branched process

3. Type III- LCs having two processes

4. Type IV- LCs having three processes

5. Type V- LCs having three or more processes with collaterals

Black arrows indicate typical dendritic CD1a$^+$ LCs and a red arrow indicates non-dendritic CD1a$^+$ LC.

Board, the sample size for this study was calculated based on data available from a previous study (Lasisi et al., 2013). The calculated sample size was 15 for each group, when the alpha error was 1%, with power of 80%. Samples were collected from patients that fulfilled the inclusion criteria after obtaining an informed consent. Normal buccal mucosal tissue samples were collected from 16 patients that underwent substitution urethroplasty for urethral stricture disease. Malignant buccal mucosal tissue samples were collected from 15 patients that underwent radical surgical resection of buccal carcinoma. They were fixed in neutral formalin, dehydrated, embedded in paraffin and then processed for immunohistochemistry. Sections with a thickness of 4-5 µm were cut, mounted on Poly-L-Lysine coated slides and stained with CD1A-EP80 Rabbit monoclonal antibody (PathnSitu, Livemore, CA, USA). Serial sections were taken, and every fourth section was used for counting. The length of basement membrane used for counting was 25mm per tissue (Cellsens image analyzing software). At 40X magnification, the CD1a+ LCs were manually identified, morphologically subtyped, counted and analyzed statistically.

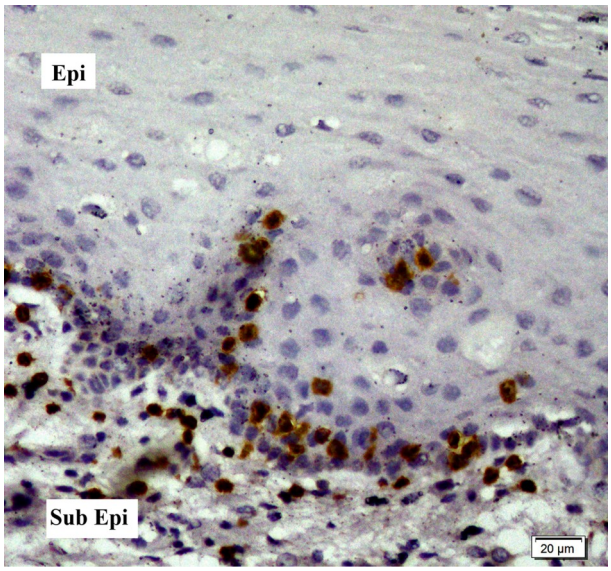


Fig 2. Non-dendritic CD1a+ LCs located in the basal layer of stratified squamous epithelium (Epi) and subepithelium (SubEpi) of normal buccal mucosa (brown cells).

RESULTS

All 16 patients in the normal group were men, while there were 10 men and 5 women in the malignant group. The mean age of patients in the normal group was 38.19±9.75 and in the malignant group was 56.87±8.95.

All five subtypes of LCs as described by Figueroa & Caorsi (Figueroa and Caorsi, 1980) were identified using CD1a+ immunomarker (Fig. 1a,b). In addition to the previously described five subtypes, a new subtype of CD1a+ polygonal cells without any dendritic processes were identified, which were categorized as “non-dendritic LCs”. These cells were predominantly located along the basal layer of epithelium (Fig. 2). The five described subtypes of CD1a+ LCs were categorized as “typical LCs”, being predominantly located along the suprabasal layer of the epithelium.

The overall mean number of CD1a+ LCs in the normal and malignant groups were 27.68±4.66 and 25.46±8.76 respectively per mm length of basement membrane. In the normal group, the mean number of typical dendritic and non-dendritic CD1a+ LCs were 10.58±4.79 and 17.11±4.43 respectively. In the malignant group, the mean num-

Table 1. Comparison of mean number of CD1a+LCs in normal and malignant buccal mucosa using Student's t-test.

Categories		Typical dendritic LCs*	Non-dendritic LCs*	p-value	Overall LCs*
Normal (n=16)	Median (Range)	9.96 (3.64-22.08)	15.5 (11.2-25.16)	<0.001	27.87 (19.48-33.88)
Malignant (n=16)	Median (Range)	9.48 (0.4-25.48)	13.32 (4.48-19.60)	0.49	23.26 (4.52-45.08)
p-value		0.92	0.004		0.14

*per mm length of basement membrane

Table 2. Comparison of overall mean number of CD1a+LCs in normal and malignant groups against the relevant age subgroups using the Student's t-test.

Groups	Age	Mean values*	p-value
Normal tissues (n16)	≤45yrs	27.16±4.90	0.586
	>45yrs	28.54±4.52	
Malignant tissues (n15)	≤60yrs	27.00±7.72	0.069
	>60yrs	20.18±10.66	

*per mm length of basement membrane

Table 3. Comparison of the mean number of non-dendritic CD1a+LCs in both normal and malignant groups combined against age using the Student's t-test.

Variable	Age	Buccal mucosa(n=31)*	p-value
Mean number of non-dendritic LCs	≤60yrs	15.83±4.63	0.026
	>60yrs	11.29±3.83	

*per mm length of basement membrane
Pearson's correlation test showed a negative correlation between the age and the overall mean number of LCs but failed to reach statistical significance.

ber of typical dendritic and non-dendritic CD1a+ LCs were 10.79±7.81 and 12.34±3.99 respectively. A significantly higher number of non-dendritic LCs were seen in the normal group, while this difference in the malignant group was not statistically significant (Table 1).

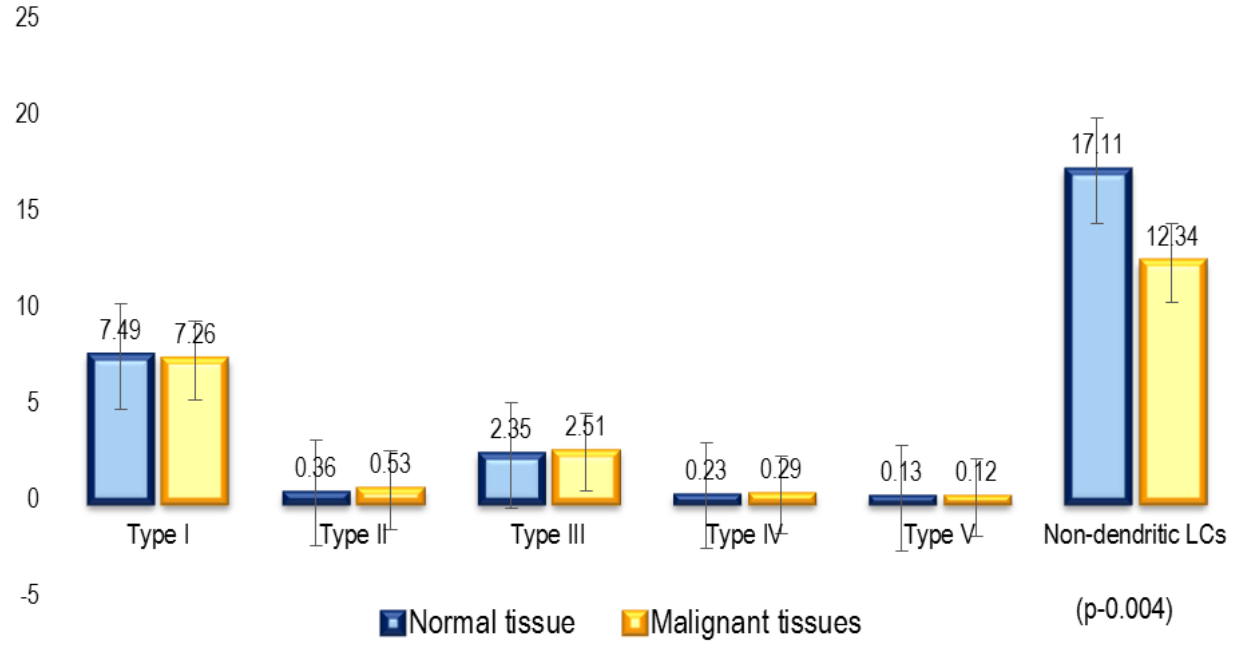
The number of typical LCs distributed in each subtype was comparable across both the normal and malignant groups. However, the number of non-dendritic LCs present in the malignant group were significantly fewer (p-0.004) (Chart 1).

The age-group of patients in the normal group was lower than those in the malignant group, hence they were further sub classified as ≤45 and >45yrs, ≤60 and >60yrs respectively. There was no significant difference in the overall mean number of CD1a+ LCs in both age-based subgroups (Table 2). The mean number of the non-dendritic CD1a+ LCs was significantly higher in younger patients (≤60yrs) (Table 3).

DISCUSSION

Oral mucosal LCs are important mediators of mucosal immune responses (Williamson et al., 2002; Stumbles et al., 2003; Mowat, 2005), and

Chart 1. Comparison of individual types of CD1a+ LCs between the normal and malignant groups.



play a vital role in the defence mechanism against microbial infections and epithelial tumours of the oral cavity. The remarkable diversity in the function of LCs enables the mucosal immune system to mount an appropriate immunological response (Ma et al., 2013). The flexibility and dynamics of this system is attributed to the diverse subsets of LCs (Reinartz et al., 2016), which are independent in their maturation, lineage of origin and function (Sato, 2007). LCs have the ability to nullify transepithelial attacks by invading pathogens, irritants and allergens (Kosten et al., 2017).

In the present study, two categories of CD1a+ LCs were identified in both normal and malignant buccal mucosa: a) typical dendritic Langerhans cells and b) non-dendritic Langerhans cells (a new entity). The typical dendritic LCs have been well described and classified into five sub-types based on the number of dendritic processes and their branching pattern (Figueroa and Caorsi, 1980; Upadhyay et al., 2013). The predominant suprabasal location of these cells observed in this study was also noted by Negishi and Yamaguchi (2007). They reported a higher number of S-100 positive LCs in the suprabasal layer of buccal mucosa.

The non-dendritic LCs are polygonal or rounded in shape, with a well-defined nucleus, showing no signs of degeneration. They had no dendritic processes and were located predominantly in the basal layer. This is the first reported description of CD1a+ non-dendritic LCs in the normal buccal mucosa, to the best of our knowledge. LCs without dendritic processes have been described in other tissues using the Zinc-Iodide osmium method, including human exocervical epithelium (Rabi et al., 2014), human palatine tonsil (Indrasingh et al., 2002) and labial epithelium of Bonnet monkey (Koshy et al., 2003). Rani et al. (2015) described S100 positive non-dendritic cells in poorly differentiated squamous cell carcinoma of buccal mucosa and called them abnormal cells. Breathnach et al. (1977) described two types of LCs in the epidermis of normal skin using electron-microscopy: a) Type I cells were classically dendritic in shape, had numerous Langerhans (Birbeck) granules and were located in the suprabasal layer of the epithelium; b) Type II cells were not classical in shape, had fewer dendrites, fewer Langerhans (Birbeck) granules, with more mitochondria, an electron-dense cytoplasm and were located primarily along the basal layer (Breathnach et. al 1977). The CD1a+ non-dendritic LCs demonstrated in this study could belong to a separate category of non-classical LCs which may have a unique role in human physiology and immunological response to pathogens.

Previous reports suggest huge variation in the number of CD1a+LCs between individuals (Daniels, 1984), and also between different areas of oral mucosa in the same individual (Negishi and Yamaguchi, 2007). Factors influencing the number of LCs present in the buccal mucosa could include- a) location within the oral cavity, b) embryological origin and c) mobility of the mucosa (Negishi and Yamaguchi, 2007).

Several authors have demonstrated a higher number of S100 positive cells in the normal buccal mucosa compared to other sites of the oral cavity (Daniels, 1984; Cruchley et al., 1989; Negishi and Yamaguchi, 2007). Concurrent with the above finding, the total number of CD1a+LCs ranged from 19.48 to 33.88 in this study.

A higher number of CD1a+ LCs was demonstrated in oral lichen planus when compared to normal mucosa (Kumar et al., 2019). In oral submucous fibrosis, number of CD1a+ LCs were reported to be significantly higher than normal tissues, and progressively increased with the grade of disease (Narayanan and Narasimhan, 2015). Certain reports have suggested a significant decrease in the number of CD1a+LCs in malignant tissues when compared to normal (Angadi and Krishnapillai, 2012). Langerhans cells have been studied in oral premalignant lesions and oral carcinoma using various markers like CD1a, phosphatase and tensin homolog (PTEN), CD83 and argyrophilic protein nucleolar organiser regions (AgNOR) with varying results, thus making them less reliable when used in a clinical setting (Kinoshita et al., 1996; Perez et al., 2005; Angadi and Krishnapillai, 2012; Gomes et al., 2016). It could be postulated that in premalignant lesions, a surge of inflammatory markers could increase the number of CD1a+LCs, while in malignant tissue, the tumor cells may evade identification by the antigen presenting cells by modifying their microenvironment, resulting in a diminished number of CD1a+LCs.

In the malignant group, huge variations in the number of LCs were noted between patients. Few studies have shown a decrease in the number of LCs in malignant oral mucosa compared to normal (Arachchi et al., 1989; Upadhyay et al., 2012; Lasisi et al., 2013). However, there are other studies suggesting higher numbers of CD1a+ LCs in oral squamous cell carcinoma (Cruchley et al., 1994). Upadhyay et al. (2012) demonstrated an increase in the number of CD1a+ LCs in malignant lesions as compared to dysplastic lesions. Few others have reported an increase in the number of S100+ LCs in malignant oral mucosa compared to normal (Kurihara and Hashimoto, 1985; Rani et al., 2015). Huge variation in numbers in the malignant group was also noted in our study, 3 patients had a few CD1a+LCs (4.88-11.76) while 4 had higher numbers (31.8-45.08). Although the overall number of CD1a+ LCs was comparable between both groups, the number of non-dendritic LCs were significantly lesser (p-0.004) in the malignant group. This could represent an altered immunological response in malignant tissues.

There are reports that suggest a higher number of CD1a+LCs in mobile areas of oral mucosa com-

pared to immobile areas of oral mucosa, like the hard palate and gingivobuccal sulcus. Keratinization and endodermal origin of the immobile areas in oral mucosa are thought to be responsible for the lower number of LCs (Negishi and Yamaguchi, 2007).

Evidence in literature suggests a decrease in the number of LCs with increasing age, in skin (Gilchrest et al., 1982). In murine skin, an age-related decrease in dermal LC density and impairment of their phagocytic ability has been demonstrated (Xu et al., 2012). In the buccal mucosa, Cruchley et al. (1994) did not find a significant change in the number of LCs with increase in age. In keeping with the above finding, the current study showed no significant reduction in the overall number of CD1a+ LCs with increasing age. In the malignant group, the decrease noted in the number of typical dendritic LCs with increasing age did not reach significance. However, a statistically significant reduction in the number of non-dendritic CD1a+ LCs was noted with advancing age, in both groups.

Limitations of the study would include the small sample size. The findings of this study provide direction for future research to further characterize the physiology and immunological role of non-dendritic LCs.

In conclusion, non-dendritic CD1a+LCs are present in normal and malignant buccal mucosa. Significantly fewer number of non-dendritic LCs found in malignant tissues and older individuals could possibly explain their altered immunological response, and increased vulnerability to the attacking pathogens and carcinogens. Further studies evaluating methods to increase the number of non -dendritic cells, augment their immunological potential, and study the resultant effect on cancer prevention and treatment could have far reaching clinical impact.

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REFERENCES

AKIHIDE NEGISHI KM, YAMAGUCHI T (2007) Histopathological study on the distribution of Langerhans cells in oral mucosa. Kitakanto Med Journal, 57: 307-310.

ANGADI PV, KRISHNAPILLAI R (2012) Evaluation of PTEN immunoexpression in oral submucous fibrosis: role in pathogenesis and malignant transformation. Head and Neck Pathol, 6(3): 314-321.

BREATHNACH AS (1977) Variations in ultrastructural appearance of Langerhans cells of normal human epidermis. Br J Dermatol, 97: 14-14.

CRUCHLEY AT, WILLIAMS DM, FARTHING PM, LESCH CA, SQUIER CA (1989) Regional variation in Langerhans cell distribution and density in normal human oral mucosa determined using monoclonal antibodies against CD1, HLADR, HLADQ and HLADP. J Oral Pathol Med, 18(9): 510-516.

CRUCHLEY AT, WILLIAMS DM, FARTHING PM, SPEIGHT PM, LESCH CA, SQUIER CA (1994) Langerhans cell density in normal human oral mucosa and skin: relationship to age, smoking and alcohol consumption. J Oral Pathol Med, 23(2): 55-59.

DANIELS TE (1984) Human mucosal Langerhans cells: postmortem identification of regional variations in oral mucosa. J Invest Dermatol, 82(1): 21-24.

FIGUEROA CD, CAORSI I (1980) Ultrastructural and morphometric study of the Langerhans cell in the normal human exocervix. J Anat, 131(Pt 4): 669-682.

GILCHREST BA, MURPHY GF, SOTER NA (1982) Effect of chronologic aging and ultraviolet irradiation on Langerhans cells in human epidermis. J Invest Dermatol, 79(2): 85-88.

GOMES JO, DE VASCONCELOS CARVALHO M, FONSECA FP, GONDAK RO, LOPES MA, VARGAS PA (2016) CD1a+ and CD83+ Langerhans cells are reduced in lower lip squamous cell carcinoma. J Oral Pathol Med, 45(6): 433-439.

INDRASINGH I, CHANDI G, VETTIVEL S (2002) Light and electron microscopic appearance of Langerhans cell (LC) in human tonsil surface epithelium: A zinc iodide osmium (ZIO) study. J Anat Soc India, 51: 156-158.

KATSUAKI SATO SF (2007) Dendritic cells- Nature and Classification. Allergology Int, 56: 183-191.

KINOSHITA Y, DOHI M, MIZUTANI N, IKEDA A (1996) Effects of preoperative radiation and chemotherapy on AgNOR counts in oral squamous cell carcinoma. J Oral Maxillofac Surg, 54(3): 304-307.

KOSHY S, INDRASINGH I, VETTIVEL S (2003) Dendritic cells in the tongue and oesophagus of laboratory guinea pig, rat, and rabbit: a light microscopic zinc iodide-osmium study. Eur J Anat, 7(1): 9-14.

KOSTEN IJ, VAN DE VEN R, THON M, GIBBS S, DE GRUIJL TD (2017) Comparative phenotypic and functional analysis of migratory dendritic cell subsets from human oral mucosa and skin. PLoS ONE, 12(7): e0180333.

KUMAR TA, VEERAVARMAL V, NIRMAL RM, AMSAVENI R, NASSAR MHM, KESAVAN G (2019) Expression of cluster of differentiation 1a-positive Langerhans cells in oral lichen planus. Indian J Dermatol, 64(1): 41-46.

KURIHARA K, HASHIMOTO N (1985) The pathological significance of Langerhans cells in oral cancer. J Oral Pathol, 14(4): 289-298.

LASISI T, OLUWASOLA AO, LASISI OA, AKANG EE (2013) Association between Langerhans cells population and histological grade of oral squamous cell carcinoma. J Oral Maxillofac Pathol, 17(3): 329-333.

MA Y, SHURIN GV, PEIYUAN Z, SHURIN MR (2013)

Dendritic cells in the cancer microenvironment. J Cancer, 4(1): 36-44.

MOWAT AM (2005) Dendritic cells and immune responses to orally administered antigens. Vaccine, 23 (15): 1797-1799.

NARAYANAN B, NARASIMHAN M (2015) Langerhans cell expression in oral submucous fibrosis: an immunohistochemical analysis. J Clin Diagn Res JCDR, 9(7): ZC39-ZC41.

PEREZ L, SHURIN MR, COLLINS B, KOGAN D, TOURKOVA IL, SHURIN GV (2005) Comparative analysis of CD1a, S-100, CD83, and CD11c human dendritic cells in normal, premalignant, and malignant tissues. Histol Histopathol, (4): 1165-1172.

PITIGALA-ARACHCHI A, CRANE IJ, SCULLY C, PRIME SS (1989) Epithelial dendritic cells in pathological human oral tissues. J Oral Pathol Med, 18(1): 11-16.

RABI S, LIONEL J, INDRASINGH I (2014) Morphological study of dendritic cells in human cervix by zinc iodide osmium method. J Clin Diagn Res, 8(6): AC01-AC04.

RANI SV, ARAVINDHA B, LEENA S, BALACHANDER N, MALATHI LK, MASTHAN MK (2015) Role of abnormal Langerhans cells in oral epithelial dysplasia and oral squamous cell carcinoma: A pilot study. J Nat Sci Biol Med, 6(Suppl 1): 28-33.

REINARTZ SM, VAN TONGEREN J, VAN EGMOND D, DE GROOT EJ, FOKKENS WJ, VAN DRUNEN CM (2016) Dendritic cell subsets in oral mucosa of allergic and healthy subjects. PLoS ONE, 11(5): e0154409.

STEINMAN RM (1991) The dendritic cell system and its role in immunogenicity. Ann Rev Immunol, 9(1): 271-296.

STEINMAN RM, COHN ZA (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. J Exper Med, 137(5): 1142-1162.

STUMBLES PA, UPHAM JW, HOLT PG (2003) Airway dendritic cells: co-ordinators of immunological homeostasis and immunity in the respiratory tract. APMIS, 111(7-8):741-755.

TOEWS GB, BERGSTRESSER PR, STREILEIN JW (1980) Langerhans cells: sentinels of skin associated lymphoid tissue. J Invest Dermatol, 75(1): 78-82.

UPADHYAY J, RAO NN, UPADHYAY RB (2012) A comparative analysis of Langerhans cell in oral epithelial dysplasia and oral squamous cell carcinoma using antibody CD-1a. J Cancer Res Ther, 8(4): 591-597.

UPADHYAY J, UPADHYAY RB, AGRAWAL P, JAITLEY S, SHEKHAR R (2013) Langerhans cells and their role in oral mucosal diseases. North Am J Med Sci, 5(9): 505-514.

WILLIAMSON E, BILSBOROUGH JM, VINEY JL (2002) Regulation of mucosal dendritic cell function by receptor activator of NF- B (RANK)/RANK ligand interactions: impact on tolerance induction. J Immunol, 169 (7): 3606-3612.

XU YP, QI RQ, CHEN W, SHI Y, CUI ZZ, GAO XH, CHEN HD, ZHOU L, MI QS (2012) Aging affects epidermal Langerhans cell development and function and alters their miRNA gene expression profile. Aging, 4 (11): 742-754.