Original Article

# Expression of CD1a by Langerhans cells in oral mucosa of submucous fibrosis patients and arecanut/gutkha chewers

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**ABSTRACT**

**Background:** Oral submucous fibrosis (OSF) is insidious in onset and is characterized by inflammation and progressive fibrosis of the lamina propria. Previously, consumption of chillies and arecanut were thought to be the etiology of OSF. Various autoantibodies and specific human leucocyte antigens (HLA) in some patients have indicated an autoimmune role as well as a genetic predisposition for the disease. Studies have also found increased presence of Langerhans cells in OSF patients; this suspects the antigenic behaviour of arecanut on mucosa.

**Study Design:** Biopsies were taken from the 70 subjects (20 patients with OSF, 20 patients who were arecanut/gutkha chewers with no oral lesions and 30 controls) and sections were subjected to immunohistochemical staining with CD1a.

**Results:** Immunostaining exhibited brown reaction product deposited in the nucleus and cytoplasm of dendritic cells. There was significant difference in number of Langerhans cells when compared between control, arecanut chewers without any visible lesions and OSMF patients, however the number of Langerhans cells are similar in arecanut chewers and OSMF patients

**Conclusion**: Arecanut seems to exhibit an antigenic behaviour on the oral mucosa however, not all individuals who chew arecanut or gutkha present with OSF. This is probably due to the fact that these may not be the only cause for causation of OSF. Other factors such as nutrition, immunity, genetics may add on to the etiology of OSF.

**Keywords:** CD1a, langerhans cells, OSF

## INTRODUCTION

Langerhans cells (LC) are highly specialized bone marrow derived cells, situated suprabasally in most stratified squamous epithelia such as the epidermis and the epithelium of oral mucosa; including gingiva[1] These cells are not visible on routine histological staining[2] and express CD1. They are thought to act as antigen-presenting cells during induction of immune response.[3] LC are distributed throughout the nucleated layers of epidermis.

Dorsum of the tongue and buccal mucosa have the highest density of Langerhans cells per mm of epithelial surface length.[1] The number of cells in the buccal epithelium is constantly about 20% higher than in the epithelium of the tongue.[4] These cells have been thought as essential for the immune surveillance function against antigens involved in allergic reactions as well as against emergence of new antigens expressed by malignant transformation.[3]

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Increase in the number of LC has been noted in lichen planus, oral cancer, erythema multiformae, histiocytosis X, contact hypersensitivity, radicular cysts, Behcet's syndrome, aphthous ulcers[5] and oral submucous fibrosis.[2] In contrast there is decrease in number of oral Langerhans cells in HIV seropositive patients.[6]

Oral submucous fibrosis (OSF) is insidious in onset and is characterized by inflammation and progressive fibrosis of the lamina propria. The major complaints in OSF patients are progressive inability to open the mouth because of the accumulation of elastic fibrous tissue in the juxta-epithelial region of oral mucosa and muscle degeneration.[7] OSF predominantly occurs in Indians and South East Asians. [8,9,10,11,12] Cox et al (1996) estimated about 2.5 million people are affected globally. Since OSF is confined to a particular geographic region it was thought that the disease was related to dietary or cultural habits present in those regions. [13] Previously, consumption of chillies and arecanut were thought to be the etiology of OSF. [12] The high incidence of OSF in the Indian subcontinent is causally associated with the commonly prevailing habit of chewing areca nut and tobacco. Desa (1957), Rao (1962), Pindborg et al (1964), Sirsat et al (1967) found circulating and tissue eosinophilia, gammaglobulinemia, and high mast cell response in early stages of OSF. [14] Sirsat and Pindborg (1967) suggested immunological role, Canniff et al (1986) reported significantly elevated serum IgG. The role of cytotoxicity and genotoxicity of arecanut products, trace elements and cytokines in the causation of fibrosis has been well documented in literature, [15] so is the increase in collagen cross linkages caused by upregulation of lysyl oxidase by OSF fibroblast. [16, 17] At present multifactorial origin of OSF has been thought of; like nutritional deficiency, genetic susceptibility, salivary constituents, autoimmunity and collagen disorder. [10,13,18,19] However, arecanut chewing is the most accepted etiologic factor[12] Haque MF et al (1997) had found increased presence of Langerhans cells in OSF patients; this suspects the antigenic behaviour of arecanut on mucosa.[2] Hence the study was planned to find out if arecanut acts as an antigen producing antigenic reaction leading to OSF.

## MATERIAL AND METHOD:

Ethical clearance was taken from the institutional ethical committee (Kasturba medical college ethics committee, Mangalore Karnataka). 70 subjects were taken for the study which comprised of 3 groups, Group I consisted 20 patients with OSF (any clinical stage). Group II consisted of 20 patients who were arecanut/gutkha chewers with no oral lesions. Group III consisted of 30 controls i.e. subjects who do not have any chewing habits and do not have any oral lesion

Written consent were taken from the patients, and interview based questionnaire about their habit were filled. Biopsies were taken from the buccal mucosa for OSF patients. Tissue sample from arecanut/gutkha chewers and control subjects were obtained as superfluous margins from buccal flaps rose for minor oral surgery procedures.

The entire specimen were fixed in 10% neutral buffered formalin for 24-48 hrs and embedded in paraffin wax. 4-µm sections of OSF were obtained and stained with haematoxylin-eosin to confirm the clinical diagnosis. Other 3-µm sections were obtained from each block for immunohistochemical staining. Primary antibody (CD1a) and Supersensitive polymer- HRP Ready – to use kit was used manufactured by Biogenix(USA)

## RESULTS

Immunostaining exhibited brown reaction product deposited in the nucleus and cytoplasm of dendritic cell. The dendritic cells were identified based upon their morphology and staining property.

Photomicrographs were taken in 10X magnification in TIFF format by inbuilt digital camera Olympus BX41. Images were assessed in Image J software version 14.1. (National Institutes of health, USA Java 1.6.0_10). The average areas of 10 Langerhans cells were taken from each photograph which was calculated as

$3\mu m^2$. With this estimation a grid was superimposed with horizontal and vertical cube dimensions of 3µ(Fig 1). All the cells not less than $3\mu m^2$ were counted as Langerhans cell. Keeping the grid, superimposed cells were counted without repetition using cell counter. When comparing the number of Langerhans cells between 3 different groups: control, arecanut chewers without any visible lesions and OSMF patients, a highly significant difference was found between them (p=<.001). Similar is the result when comparing the number of Langerhans cells between control, arecanut chewers without any visible lesions and control and OSMF patients (p=<.001). On comparing arecanut chewers without visible lesions and OSMF patients, p= 0.786 inferring that the number of Langerhans cells are similar in arecanut chewers and OSMF patients (Table1).

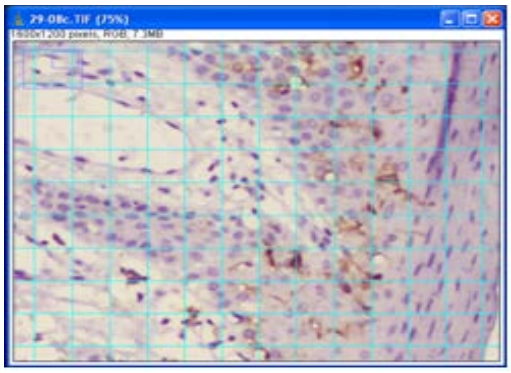

**Fig. 1-Grid superimposed for counting (using Image J software)**

**Table1**

| Comparison groups | p Value |
|---|---|
| Comparison between OSMF, control and arecanut chewers | <.001 |
| Comparison between control and arecanut chewers | <.001 |
| Comparison between control and OSMF | <.001 |
| Comparison between OSMF and arecanut chewers | .786 |

## DISCUSSION

OSF is regarded as a precancerous and potentially malignant condition. It is thought to be multifactorial in origin with the high incidence in people who chew arecanut. OSF have significant malignant transformation rate (7-30%). [20,21] Problems like burning mouth and trismus in initial stage and deafness due to fibrosis of Eustachian tube, nasal voice and dysphagia to solids in advanced stages have psychological effect on the patients. Generally young patients develop signs and symptoms of OSF within 3.5 yrs from onset of habit while in older patient it takes 6.5 yrs.[8,22] Many studies have been attempted to know the etiology of OSF. Various epidemiological studies and large cross- sectional studies have provided evidence that arecanut is main etiological factor. Specific human leukocyte antigens (HLA) in some patients have indicated genetic predisposition of the disease.[22]

It is estimated that there are at least 6 alkaloids present in betel nut amongst which arecoline, arecaidine [19] and to a smaller extent tannin [19,22,23] appears to interfere with molecular process of deposition and degradation of extracellular matrix (ECM) molecules such as collagen causing imbalance in normal process. [22] It is believed that the imbalance occurs due to reduced phagocytosis of collagen by fibroblasts, up/down regulation of lysyl oxidase, MMP (matrix metalloprotenase) and tissue inhibitors of MMP. [17,20] This process may also be influenced by upregulation of fibrogenic cytokines and downregulation of antifibrotic cytokine.[24] The components of betel quid exhibit genotoxicity and may alter the structure of DNA, proteins and lipids, resulting in the production of antigenicity.[25] It is the known fact that Langerhans cells are the sole epithelial cell population responsible for antigen processing and presentation and subsequent induction of T-cell mediated immune respone.[26,27,28] In skin and oral mucosa, both the density and functional capacity of Langerhans cells are considered critical determinants of the capacity of a given site to initiate a protective immune response to antigens.[26]

Studies have shown that LC in buccal mucosa were predominantly in a suprabasal location. Although present along the length of the epithelium they showed a tendency to cluster around the tips of connective tissue papillae. [5] In

present study, in all three groups LC were seen in the epithelium as well as in the connective tissue of the oral mucosa. The vast majority was found in the epithelium. The LC were more diffuse in the epithelium and in the connective tissue area was situated only in the area directly below the epithelium. Our finding was similar to that reported by Chiang CP et al who found a large variation in shape and size of LC which were distributed randomly in the spinous and superficial layers.

Some monoclonal antibodies recognize specific antigenic determinants of LC namely CD1a5,33,37,50,52,55,70, anti S10058,60, HLA-DR47,58,67, CD82,61,70, CD461,70 , CD361,70. CD1a has most commonly been used as it is specific for LC.[5,29,30] To assess the expression of LC in OSF and arecanut/gutkha chewers, we used monoclonal antibody CD1a which was similar to other studies in which CD1a was used for identification of Langerhans cell .[30] The result of our study using the standardized technique shows significant difference in Langerhans cells in OSF patients and control, p value being <0.001. The maximum average of Langerhans cells in OSF patients was 13 (ranging from 0-13) whereas the maximum number of Langerhans cells in control was 7 (ranging from 0-7).

However Chiang CP (1998) showed that the LC density in OSF was low in comparison with normal mucosa. The authors believe that ingredients of betel quid may have the direct toxic effect or the LC may have a diminished nutritional supply or there may be reduced recruitment of LC from circulation because of fibrosis and progressive loss of vascularity.[30]

On further segregating the number of Langerhans cells according to habit, the maximum number of Langerhans cells were seen in gutkha chewers that is 13, followed by white arecanut (ranging from 2-9) and 12 in red arecanut chewer. Also present was the statistical significant difference between number of Langerhans cells in arecanut /gutkha chewers and control. The maximum average of Langerhans cells in arecanut /gutkha chewers was 29 (ranging from 0-29). Further classifying the chewers, the maximum number of Langerhans cells i.e. 29 was present in gutkha chewers. Out of 9 beeda chewers, the number of Langerhans cells ranged from 0-24, 0-18 in white arecanut chewer and 0-4 in red arecanut chewers. However, there is no significant difference between number of Langerhans cells in OSF patients and arecanut /gutkha chewers.

The result of our study show that irrespective of the type of chewing habit (red or white arecanut, gutkha, beeda), arecanut seems to exhibit an antigenic behavior on the oral mucosal tissue.

However, literature shows that the incidence of OSF is relatively high in gutkha chewers,[10,31] it only strengthens the proposition that gutkha is not only antigenic to oral mucosal tissues but some of its components can predispose to OSF by evoking chronic inflammatory reaction. Gutkha consists mainly of areca-nut, tobacco, and flavours. It is the most commonly used commercially freeze-dried areca-nut products. Gutkha contains the nut in high concentrations and has replaced other commercially available arecanut preparation like betel quid. [21] It is also interesting to know that all gutkha chewers do not exhibit OSF. It seems that the constituents of arecanut have a genotoxic affect in genetically predisposed individuals.

Unlike gutkha, arecanut may not really have genotoxic effects and may simply stimulate the inflammatory cells. It has been seen that many individuals who chew pure arecanut do not present with OSF. This is probably due to the fact that arecanut may not be able to incite chronic inflammatory response in these individuals owing to their genetic stability or other protective factors such as nutritional status, inherent immunity, and action of suppressor T cells and the inherent ability of the body to repair genotoxicity in the fibroblasts / epithelial cells

## CONCLUSION

Our study showed gutkha is strongly associated with the development of OSF as out of 20 OSF patients, 15 were gutkha chewers. There was significantly high number of Langerhans cells in arecanut chewers compared to control so,

arecanut seems to exhibit an antigenic behaviour on the oral mucosal tissue however there is no difference in antigenic behaviour irrespective of the type of chewing habit (red or white arecanut, gutkha, beeda). In our study, few individuals who chew arecanut or gutkha do not present with OSF. This is probably due to the fact these may not be the only cause for causation of OSF. Other factors such as nutrition, immunity, genetics may add on to the etiology of OSF.

**Limitation of our study:**

Many patients who chew gutkha develop OSF in greater number than other groups, larger samples with large number of subjects in various habits (red/white arecanut, gutkha and beeda) should be included for assessing the effects of each variety on the oral mucosa.Number of Langerhans cell vary depending on the site of oral mucosa, so biopsies from various sites of oral mucosa has to be studies to form a baseline for the number of Langerhans cells in various sites.We noted difference in number of Langerhans cells in different stages of OSF in our study but our study sample consisted of only early, moderately advanced and advanced cases and only 3 cases were of early and advanced case, hence study samples representing various stages of OSF should be considered.

## REFERENCES


1. Cruchley AT, Williams DM, Farthing PM, Lesch CA, Squier CA. Regional variation in Langerhans cell distribution and density in normal human oral mucosa determined using monoclonal antibodies against CD1, HLADQ and HLADP. *J Oral Pathol Med*. 1989 Oct; 18 (9): 510-6

2. Haque MF, Harris M, Meghji S, Speight PM. An immunohistochemical study of oral submucous fibrosis. *J Oral Pathol Med*. 1997 Feb; 26 (2): 75-82

3. Lombardi T, Hauser C, Budtz-Jörgensen E. Langerhans cells: structure, function and role in oral pathological conditions. *J Oral Pathol Med*. 1993 May; 22 (5): 193-202

4. Bos IR, Burkhardt A. Interepithelial cells of the oral mucosa. Light and electron microscopic observations in germfree, specific pathogen-free and conventionalized mice. J Oral Pathol. 1980 Mar; 9 (2): 65-81

5. McCartan BE, Lamey PJ. Expression of CD1 and HLA-DR by Langerhans cells (LC) in oral lichenoid drug eruptions (LDE) and idiopathic oral lichen planus (LP). *J Oral Pathol Med*. 1997 Apr; 26 (4): 176-80.

6. Chou LL, Epstein J, Cassol SA, West DM, He W, Firth JD. Oral mucosal Langerhans cells as target, effector and vector in HIV infection. *J Oral Pathol Med*. 2000 Sep; 29 (8): 394-402.

7. Pillai R, Balaram P, Reddiar KS. Pathogenesis of oral submucous fibrosis. Relationship to risk factors associated with oral cancer. 1992 Apr 15; 69 (8): 2011-20

8. Ranganathan K, Devi MU, Joshua E, Kirankumar K, Saraswathi TR.Oral submucous fibrosis: a case-control study in Chennai, South India. *J Oral Pathol Med*. 2004 May; 33 (5): 274-7.

9. Lalli A, Tilakaratne WM, Ariyawardana A, Fitchett C, Leigh IM, Hagi-Pavli E, Cruchley AT, Parkinson EK, Teh MT, Fortune F, Waseem A. An altered keratinocyte phenotype in oral submucous fibrosis: correlation of keratin K17 expression with disease severity. *J Oral Pathol Med.* 2008 Apr; 37 (4): 211-20

10. Ahmad MS, Ali SA, Ali AS, Chaubey KK. Epidemiological and etiological study of oral submucous fibrosis among gutkha chewers of Patna, Bihar, India. *J Indian Soc Pedod Prev Dent*. 2006 Jun; 24 (2): 84-9

11. Yang YH, Lien YC, Ho PS, Chen CH, Chang JS, Cheng TC, Shieh TY. The effects of chewing areca/betel quid with and without cigarette smoking on oral submucous fibrosis and oral mucosal lesions. *Oral Dis*. 2005 Mar; 11 (2): 88-94.

12. Sinor PN, Gupta PC, Murti PR, Bhonsle RB, Daftary DK, Mehta FS, Pindborg JJ. A case-control study of oral submucous fibrosis with special reference to the etiologic role of areca nut. *J Oral Pathol Med*. 1990 Feb; 19 (2): 94-8

13. Kiran Kumar K, Saraswathi TR, Ranganathan K, Uma Devi M, Elizabeth J. Oral submucous fibrosis: a clinico-histopathological study in Chennai. *Indian J Dent Res*. 2007 Jul-Sep; 18 (3): 106-11

14. Prabhu SR, Wilson D.F, Daftary DK, Johnson NW. Oral Diseases in the Tropics. *Oxford University press*, 1992. New Delhi. 417-22

15. Rooban T, Saraswathi TR, Al Zainab FH, Devi U, Elizabeth J, Ranganathan K. A light microscopic study of fibrosis involving muscle in oral submucous fibrosis. *Indian J Dent Res*. 2005 Oct-Dec; 16 (4): 131-4

16. Tai YS, Liu BY, Wang JT, Sun A, Kwan HW, Chiang CP. Oral administration of milk from cows immunized with human intestinal bacteria leads to significant improvements of symptoms and signs in patients with oral submucous fibrosis. *J Oral Pathol Med*. 2001 Nov; 30 (10): 618-25.

17. Trivedy C, Warnakulasuriya KAAS, Hazarey VK, Tavassoli M, Sommer P, Johnson NW. The upregulation of lysyl oxidase in oral submucous fibrosis and squamous cell carcinoma. *J Oral Pathol Med.* 1999; 28: 246-51.

18. Rajendran R, Rani V, Shaikh S. Pentoxifylline therapy: a new adjunct in the treatment of oral submucous fibrosis. *Indian J Dent Res*. 2006 Oct-Dec; 17(4): 190-8

19. Shieh DH, Chiang LC, Lee CH, Yang YH, Shieh TY. Effects of arecoline, safrole, and nicotine on collagen phagocytosis by human buccal mucosal fibroblasts as a possible mechanism for oral submucous fibrosis in Taiwan. *J Oral Pathol Med*. 2004 Oct; 33 (9): 581-7

20. Aziz SR. Oral submucous fibrosis: Case report and review of diagnosis and treatment. J Oral Maxillofac Surg 2008; 66:2386-9.

21. Arakeri G, Brennan PA. Oral submucous fibrosis: an overview of the aetiology, pathogenesis, classification, and principles of management. *Br J oral Maxillofac Surg*. 51 (2013) 587–593

22. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: Review on aetiology and pathogenesis. *Oral Oncology*. 2006; 42: 561– 8

23. Angadi PV, Rao SS.arecanut in pathogenesis of oral submucous fibrosis:revisited. J Oral Maxillofac Surg 2011; 15:1-9.

24. Bishen KA, Radhakrishnan R, Satyamoorthy K. The role of basic fibroblast growth factor in oral submucous fibrosis pathogenesis. *J Oral Pathol Med.* 2008 Aug; 37 (7): 402-11

25. Jeng JH, Kuo ML, Hahn LJ, Kuo MYP. Genotoxic and Non-genotoxic Effects of Betel Quid Ingredients on Oral Mucosal Fibroblasts invitro. *J Dent Res*. May 1994; 73(5): 1043-9

26. van Loon LAJ, Krieg SR, Davidson CL, Bos JD. Quantification and distribution of lymphocyte subsets and Langerhans cells in normal human oral mucosa and skin. *J Oral Pathol Med.* 1989 Apr; 18 (4): 197-20)

27. Farthing PM, Matear P, Cruchley AT. The activation of Langerhans cells in oral lichen planus. *J Oral Pathol Med.* 1990 Feb;19(2):81-5

28. van Loon LAJ, van Elsas PW, Bos JD, ten Harkel-Hagenaar HC, Krieg SR, Davidson CL. T-lymphocyte and Langerhans cell distribution in normal and allergically induced oral mucosa in contact with nickel-containing dental alloys. *J Oral Pathol.* 1988 Mar; 17 (3): 129-37

29. Crunchley AT, Speight PM, Williams DM. Dual expression of the cell- surface antigens HLA-DR and CD1 (T6) by langerhans cells in human buccal mucosa and skin. *Arch oral Biol*. 1987; 32 (11): 849-53

30. Chiang CP, Kao CY, Liu BY, Wang JT, Lin SK, Kuo MYP. Reduction of Langerhans cell density in oral epithelium of patients with oral submucous fibrosis. *Chin Dent J* 1998 Sept; 17(3): 137-47

31. Bathi RJ, Pareveen S, Burde K. The role if gukta chewing in oral submucous fibrosis: A case-report. *Quintessence International* 2009, 40(6): e19-e25