**Original Research Article**

# Evaluation of the distribution of Langerhans cells in healthy peri-implant mucosa in comparison to that of healthy gingiva – An original research

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## Abstract

**Background:** Peri- implant diseases are mainly caused by the formation of a Biofilm on the surface of the implant. This deposition of the biofilm causes an inflammation and hence destruction of the implant supporting tissues. Though the response of the host is different in peri-implanttitis and periodontitis, the exact mechanism of the difference in the host response is not very clear till date. In the present study, the distribution of langerhans cells in healthy peri-implant mucosa (HPIM) was compared with healthy mucosa (HM).

**Materials and methods:** In the present study, 15 subjects who were non-smokers were selected. Before placing the implant the first sample of healthy mucosa was obtained and this was marked as Group I. The second sample consisted of peri-implant mucosa which was obtained while the gingival former was placed and this was considered as Group II. In order to quantitatively identify the Langerhans cells in the sample, Immunochemistry was used. To compare the distribution of cells in epithelium and lamina propria, Wilcoxon matched paired test was utilized.

**Results:** Mean number of CD1a (Langerhans cells) in epithelium and lamina propria of Group I and Group II were 25.2 ± 6.41 and 27.47 ± 10.26 and 19.27 ± 7.27 and 12.46 ± 3.04 respectively. Statistically significant difference in the number of cells in epithelium and lamina propria of Group I and Group II was noted. (P=0.001)

**Conclusion:** In the lamina propria of HPIM, a lesser number of CD1a+ (Langerhans cells) were noticed compared to HM.

## Key words

Peri-implant mucosa, Peri-implantitis, Titanium implants, Langerhans cells, CD1a+, Lamina propria, Epithelium.

## Introduction

A so called cluster of "contemporary" oral infections in humans are known to comprise of Peri-implant diseases which have been known to emerge due to the routine application of osseointegrated dental implants in dental clinical practice. These peri-implant diseases have been characterized by inflammatory destruction of the supporting tissues surrounding the implant because of the formation of a biofilm on the surface of the implant [1].

Antigens and Lymphocytes have been the main focus of immunology, but the mere presence of these two alone does not lead to immunity. The dendritic cell system (DCs) and Antigen presenting Cells (APCs) are a third party of calls that form the initiators and the modulators of immunological response [2]. It has been previously noticed that dendritic cells are highly potent antigen presenting cells. It has also been observed that they are the only cells capable of initiating adaptive immune response. Langerhans cells (LCs) are the epithelial residents of Dendritic cells and serve as the sentinels of the mucosa. The alter the immune system to entry of pathogens, commensal microbes and also self-antigen tolerance. Oral LCs are capable of engaging and internalising a variety of pathogens. In the early reports, it has been found that LCs present antigens to both cytotoxic T cells and helper cells and their removal eliminates the stimulatory effect [3].

LCs form a peripheral outpost of the immune system. They also provide immunosurveillant competence to the ectodermal tissues, including epithelia of the oral mucosa. They also represent the major APC in the oral mucosal epithelium and are most commonly located in the suprabasal layer of the gingival epithelium [4]. The initiation of T-cell immunity is rather demanding. Initially, peptides from infected cells located anywhere in the body must be found and recognized by T cells that circulate in the blood stream. The amounts of specific antigen–MHC complexes on infected cells are typically small (one hundred or less per cell), and must be recognized by rare T-cell clones (usually at a frequency of 1/100,000 or less) through a TCR that has a low affinity (1 mM or less). Moreover, infected cells and tumors frequently lack the co-stimulatory molecules that drive clonal expansion of the T cell, the production of cytokines, and development into killer cells [4, 5]. In the peri-implant tissues, S-100+ LCs have been described both within the epithelium and in the lamina propria. CD1a+ LCs have been observed more frequently in the oral epithelium (basal and parabasal layers) than in the sulcular/ junctional epithelium, without any differences between peri-implantitis, healthy peri-implant tissues, and aggressive periodontitis [6]. The aim of present study was to evaluate the distribution of LCs in healthy peri-implant mucosa (HPIM) and compared to healthy mucosa (HM).

## Materials and methods

15 non-smoker subjects who were systemically healthy were selected from outpatient Department of Periodontics, Kamineni Institute of Dental Sciences. The study was approved by

Institutional Ethical Committee. The exclusion criteria consisted of the subjects who reported with systemic illness that could affect the outcome of periodontal therapy, individuals who reported with allergies to medications, pregnant or lactating women, patients using tobacco in any form, individuals with poor oral hygiene. Prior to the placement of the implant, the first sample of HM was obtained and considered as Group I. At the time of implant exposure prior to placement of the gingival, the second sample was collected and considered as Group II.

**Immunohistochemical staining**

Random selection of the samples was done. Samples that were selected, were fixed, processed and embedded in Paraffin wax. With the help of a Rotary microtome they were sectioned to the thickness of 3µm. All the sections were taken onto super frost glass slides. They were then placed in the hot air oven at 100º C for 10 min for deparaffinisation and were then taken through two changes of xylene, absolute alcohol, 70% alcohol, and 80% for 5 minutes each for rehydration. The slides were then placed under running water for 2 to 4 minutes.

**Retrieval of the Antigen**

The slides were placed in buffered saline solution and placed in a microwave oven and heated at 100ºC temperature for 5 minutes each and were then allowed to cool to room temperature prior to immunohistochemical staining. The sections were then washed with phosphate buffer solution (PBS) 3-4 times and excess of buffer was tapped off. After covering the sections with Peroxide block for 15-20 minutes, they were washed gently with PBS 3-4times. The sections were then covered with power block for 15-20mins, after tapping the excess buffer.

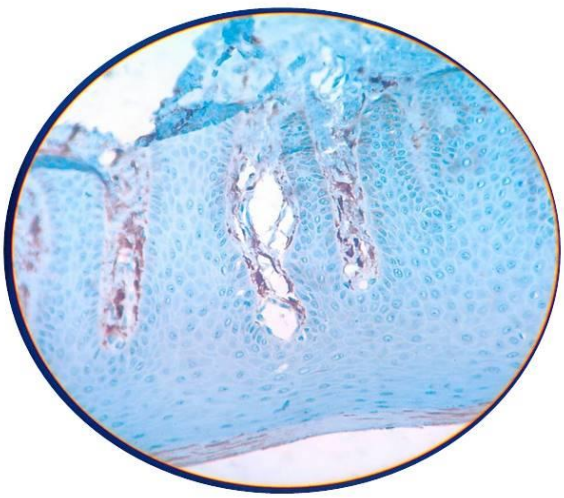
Immunohistochemistry was used to quantify the number of Langerhans cells (LCs)using CD1a as primary antibody and dendritic cells (DCs) using factor XIIIa as antibody and incubated for 1 hour at room temperature and then washed gently with PBS 3-4 times for 2 minutes each.

Super enhancer was added to the tissue sections and left for 30 minutes followed by gentle washing with PBS 3-4 times for 2 minutes each. After tapping off the excess buffer, the tissue sections were then incubated with secondary antibody for about 30 minutes and washed with PBS three to four times for 2minutes each and covered with freshly prepared substrate chromogen solution for 10 minutes followed by washing gently with distilled water for 2 minutes. The sections were then immersed in Harri's hematoxylin for 2 minutes and washed under running tap water for bluing. Finally dehydrated through series of absolute alcohol, 70% alcohol, 80% alcohol for 5 minutes each. Then the tissue sections were immersed in xylene for final clearing and later mounted by using DPX.

**Interpretation of results**

Positive immunoreactivity was indicated by the presence of brown colored end product at the site of target antigen. The cytoplasm and nucleus of cells present in the tissue sections were stained positive by the specific antibodies used.

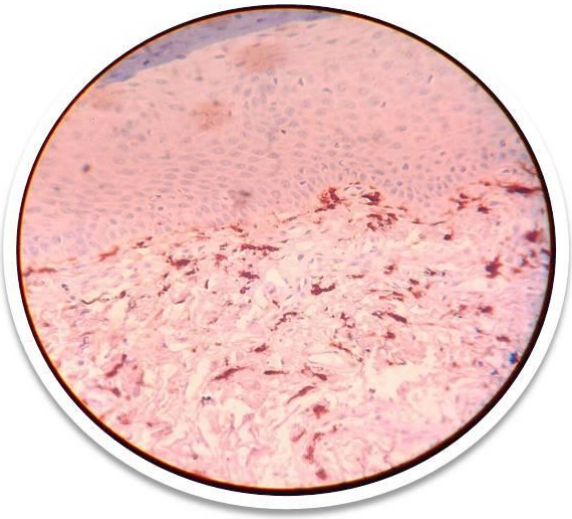
**Figure - 1:** Factor CD1a (Langerhans cells) Healthy Mucosa (HM).



Histomorphometric quantification procedure was performed for Langerhans cells by counting the cells that were CD1a positive in HM (**Figure - 1**) and HPIM (**Figure 2**). The Langerhans cells were based on nucleic and cytoplasmic staining

and their dendritic shape. Counts of cells of CD1a+ were restricted to immunolabeled cells exhibiting both the criteria that is, well-defined cell nucleus and cell body with at least two well-visualized dendrites. The cells were counted in 5 fields, which were randomly selected under 100 x magnifications. i.e. epithelium and lamina propria of each slide.

**Figure - 2:** Factor CD1a (Langerhans cells) Healthy Peri implant mucosa (HPIM).



Statistical Analysis was done by Wilcoxon matched paired test to compare the distribution of CD1a (Langerhans cells), in both epithelium and lamina propria in Group I and Group II.

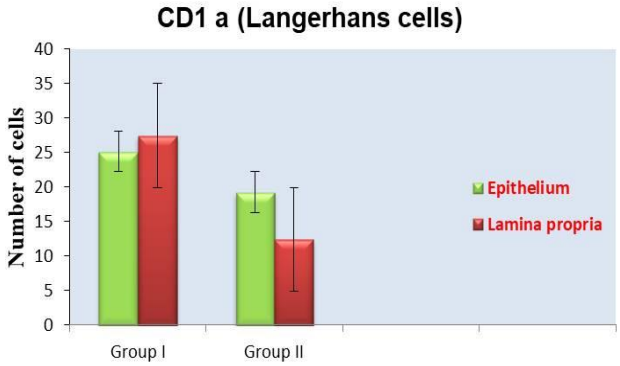
## Results

A total of 15 subjects, 6 Males and 9 Females, with mean age of 32.51 years were included in the study. The number of CD1a in epithelium and lamina propria of Group I and Group II were 25.2 ± 6.41, 27.47 ± 10.26, 19.27 ± 7.27 and 12.46 ± 3.04 respectively. Statistically significant difference in mean number of cells in epithelium and lamina propria between Group I and Group II was noted. (P=0001 and P=0.001). Though a positive relation was found in the cells in epithelium and lamina propria in Group I, there was no statistically significant difference (P=0.470), but a significant difference was observed in Group II. (P=0.023) (**Table - 1** and **Graph - 1**).

**Table - 1:** Comparison of distribution of Langerhans cells (CD1a) in Epithelium and Lamina Propria in Group I and Group II by Wilcoxon matched pairs test.

| | **Epithelium** | **Lamina propria** | **P value** |
|---|---|---|---|
| **Group I** | 25.20 ± 6.41 | 27.47 ± 10.26 | P= 0.470 |
| **group II** | 19.27 ± 7.27 | 12.46 ± 3.04 | P= 0.023* |
| **Group I Vs group II** | P = 0.001* | P = 0.001* | |

**Graph - 1:** Comparison of distribution of Langerhans cells (CD1a) in Epithelium and Lamina Propria in Group I and Group II.




## Discussion

One of the most common bone diseases in humans are the Periodontal diseases and they have a significant consequences both medically and economically [7]. They are also considered as a risk factor for some of the life-threatening medical conditions like chronic obstructive pulmonary disease, diabetes, cardiac disease [8]. For disease initiation, the presence of periodontal pathogen that is not the only factor required. The presence of periodontal pathogens alone does not lead to periodontitis. Host immune response is a central factor in the periodontal tissue destruction [9].

The innate immune system monitors the accumulation of bacterial plaque in the periodontal environment [10]. This innate immune system consists of anatomical barriers, secretory molecules, and cellular elements such

as neutrophils and macrophages [11]. The activity of innate immune cells is extremely restricted in terms of its specificity to the pathogen. Hence, in parallel, the development of a highly specific adaptive immunity takes place [12]. T and B lymphocytes which mainly represent the adaptive immune cells, invade the infected oral tissue after a few days. Then they efficiently cause pathogen clearance and provide long-lasting immunological memory [13, 14]. The immune cells not only play a protective role, but also confer to the periodontal disease pathogenesis. Neutrophils, which play a major role in bacterial clearance, accumulate in periodontal tissues and precipitate in destruction of periodontal tissue [15]. Natural killer (NK) cells are an important effector cell of the innate immune response. They have been shown to aggravate periodontitis by secreting TNF-α following direct binding to oral pathogen. TNF-α is a central cytokine critically involved in the pathogenesis of periodontitis [16].

In humans, expression of langerin and CD1a (Langerhans cells) can be employed for identification. Similar to the skin epidermis, the oral epithelium consists Langerhans cells (LCs), a unique subset of DCs expressing the C-type lectin Langerin/CD207. In mice, LCs can be easily identified by expression of CD11c, MHC class II, and Ep-CAM in addition to langerin [17]. It should also be noted that the frequency of LCs in the gingival epithelium is relatively low in comparison with the buccal mucosa which resembles the skin [18]. The LCs are not equally distributed in the gingival epithelium, as high numbers of LCs are found in the sulcular epithelium, while LCs are rarely found in the junctional epithelium [19]. Humans have about 109 epidermal LCs, the immature Dendritic cells of the skin that are located above the basal layer of proliferating keratinocytes. Freshly isolated LCs are weak T-cell stimulators, have few MHC- and accessory- molecules, but many antigen-capturing Fcg and Fce receptors. This phenotype changes dramatically within a day of culture, the cells undergo extensive transformation, antigen-capturing devices disappear, and T-cell stimulatory functions increase [20]. The situation is similar in vivo. When they encounter a powerful immunological stimulus, for example a contact allergen or a transplant, most of the LCs from the epidermis mature and move into dermal lymphatics in search of antigen-specific T cells. Small numbers of antigen-capturing DCs can also be isolated from blood, lung, spleen, heart, kidney, and the B- and T-cell areas of tonsils; these cells lack LC-specific markers (Ecadherin, Birbeck granules, Lag-1), but they also acquire accessory molecules within 1–2 days of culture, before any encounter with T cells [21, 22].

According to Norikatsu Mizumoto, et al., LCs express CD1a molecules at exceptionally high levels with virtually no detectable CD1b and only modest CD1c expression. So, in this present study CD1a marker was used because of its effectiveness in identification of Langerhans cells. It is a specific marker for Langerhans cells [23]. According to Robert E. Hunger, et al.; Two other features of LCs are intriguing in the context of their antigen-presenting role. First, LCs were shown to express high levels of CD1a, a member of the family of group 1 CD1 proteins (CD1a, CD1b, and CD1c), which share the capacity to present microbial lipid antigens to T cells [24]. Second, LCs are the only cells known to express Langerin (CD207), a C-type lectin that is sufficient to induce the formation of Birbeck granules, pentilaminar endosomal structures specific to LCs. These unique features of LCs suggested that they may be specialized by their expression of Langerin to capture particular antigens as they enter the epidermal layers, and that at least some of these antigens may be presented to CD1a-restricted T cells. The present study showed that the lamina propria of the HPIM exhibited a lower number of CD1a (Langerhans cells) (LCs) but a higher number of factor XIIIa (DCs) compared to that of the HG. This difference may explain the different immune responses between gingival and peri-implant tissues. CD1a (Langerhans cells) is a marker for LCs, which are efficient antigen-capturing immature DCs. In the peri-implant mucosa, CD1a (Langerhans cells) cells were

detected in the oral epithelium, sulcular / junctional epithelia, and lamina propria.

According To Bullon, et al. [25]; CD1a (Langerhans cells)-positive cells more frequently present in the Oral epithelium than in the sulcular–junctional (S–J) epithelium. This study is also in accordance with Marchetti, et al. [26] who conducted a study to evaluate Immunocytochemical and ultrastructural features of Peri-implant mucosa and concluded that the presence of non-keratinocyte cells; that is Langerhans cells, melanocytes, and merkel cells. In addition, the present study revealed significantly fewer CD1a (Langerhans cells) in the lamina propria of the HPIM compared to that of the gingiva. This difference seems to be maintained after disease establishment, since a reduced number of CD1a (Langerhans cells) were also described for peri-implantitis relative to the periodontitis.

In the present study, the lower number of CD1a (Langerhans cells) in the peri-implant tissue may result in reduced stimulation of the innate and acquired immune responses and a higher inflammatory reaction as part of a mechanism to control the infection in peri-implant tissue. Consistent with this hypothesis, larger proportions of neutrophil granulocytes and macrophages have been observed for peri-implantitis relative to periodontitis. This study is also in accordance with Berglund, et al. who conducted a study and concluded that, Plasma cells and lymphocytes dominated among cells in both types of lesions, i.e. Peri-implantitis and periodontitis, whereas neutrophil granulocytes and macrophages occurred in larger relative proportions in peri-implantitis than in periodontitis [27]. The stronger inflammatory response may render the peri-implant mucosal tissue more prone to destruction, which would explain the more pronounced tissue destruction in the connective tissue around the implants than in the teeth following ligature-induced diseases.

## Conclusion

In the lamina propria of HPIM, a lesser number of CD1a+ (Langerhans cells) were noticed compared to HM. Considering the drawbacks of the present study, such as sample size in each group, identification and accuracy in counting number of Langerhans cells, and the time duration i.e. this duration is insufficient for the peri-implant mucosa to have the greatest number of LCs, further studies are needed to understand in detail about many features of LCs to allow successful manipulation of the immune system.

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