Distribution of dendritic cells and langerhans cells in peri-implant mucosa

Formation of biofilm on the implant surface mainly results in peri-implant diseases which are characterized by the inflammatory destruction of the implant supporting tissues. How the host responses to infection varies amongst peri-implantitis and periodontitis, but the mechanisms that underlie the differences in responses are not clear till date. In the present study, the dendritic cell subpopulations and langerhans cells distribution was compared in healthy peri-implant mucosa (HPIM) and healthy mucosa (HM).

Methods and Material: Total of 15 non smoker subjects was selected for the study. First sample of healthy mucosa was obtained prior to the implant placement (Group I) and second sample of peri- implant mucosa was obtained at the time of placement of gingival former (Group II). Immunochemistry was used to quantify dendritic cells and langerhans cells in the samples. Statistical analysis used: Wilcoxon matched paired test was done to compare the cell distribution in epithelium and lamina propria.

Results: Mean number of CD1a (Langerhans cells) in the epithelium and lamina propria of Group I and Group II were 25. 2 ± 6. 41 and 27. 47 ± 10. 26 & 19. 27 ± 7. 27 and 12. 46 ± 3. 04 respectively. Mean number of Factor XIIIa (Dendritic cells) in epithelium and lamina propria in Group I and Group II were 30. 37 ± 5. 42 and 86. 93 ± 13. 99 and 50. 47 ± 7. 27 and 124. 33 ±10. 27 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 and P= 0. 001)

A lesser CD1a+ (Langerhans cells)number and a higher Factor XIIIa+ (Dendritic cells) number were observed in HPIM lamina propria compared to HM.

Immunology focused mainly on antigens and lymphocytes, but presence of these two entities does not always confer immunity. The dendritic cell (DCs) system of antigen presenting cells (APCs) , a third entity are the initiators and modulators of immune response.

DCs are considered efficient stimulators of T and B lymphocytes. B cells which are the antibody-secreting cell precursors, have a capacity to recognize native antigen directly through their B-cell receptors. The antigen needs to be processed and presented to T lymphocytes by Antigen Presenting Cells. Intracellular antigens, cut into peptides in the cytosol of the APCs and then tend to bind to MHC class I molecules which are recognized by CTLs, that can directly kill a target cell.

Peri-implant diseases are a group of “ contemporary” oral infections which has emerged as a outcome of increased rate of osseointegrated dental implants in clinical practice, that are mainly characterized by inflammatory destruction of the supporting tissues around the placed implant, as a result of formation of biofilm on the implant surface.

Major APCs in the oral mucosal epithelium are LCs and their location is in the suprabasal layer of gingival epithelium. These cells represent a central colony of the immune system. In contrast, Immature DCs, are less effective in initiating immunity but are specialized in capturing and processing antigens to form MHC peptide complexes. Thus, two important functions of

DCs are that they initially hold antigens and next, as mature DCs, stimulate T cells.

Both LCs and interstitial dendritic cells (IDCs) are found in gingival tissue. CD1a+ LCs have been seen more often in oral epithelium, than in sulcular or junctional epithelium, in healthy peri-implant tissues, peri-implantitis, and aggressive periodontitis. The aim of present study was to evaluate the distribution of DCs and LCs in healthy peri-implant mucosa (HPIM) and compared to healthy mucosa (HM).

Subjects and Methods:

A total of 15 subjects who were non smokers and systemically healthy subjects were selected from outpatient department of Periodontics. The study was approved by institutional ethical committee. (KIDS/IEC/2013/28) Subjects whose systemic illness known to affect the outcome of periodontal therapy, individuals allergic to medications, pregnant or lactating women, patients using tobacco in any form, individuals with unacceptable oral hygiene were excluded from the study. The first sample of HM (Group I) was obtained prior to the implant placement and the second sample of HPIM (Group II) was obtained at the time of implant exposure prior to placement of gingival former.

Immunohistochemical staining procedure:

Samples were randomly selected, fixed, processed and embedded in Paraffin wax. Later, the samples were sectioned to the 3µm thickness with Rotary microtome. All sections were taken onto super frost glass slides, and then placed in hot air oven at 100º C for 10 mins for the process of

deparaffinisation and were rehydrated by taking through two changes of xylene, absolute alcohol, 70% alcohol, 80% alcohol each for 5 minutes. The slides were placed under running tap water for a period of 2 to 5 minutes.

Antigen retrieval:

The slides were placed in Tris buffered saline solution and kept in a microwave oven and are heated for 4-5times at 100ºC temperature for 5 minutes each. The heated slides were then allowed to cool to room temperature prior to the procedure of immunohistochemical staining. The sections were later washed with phosphate buffer solution (PBS) 3-4times for 2minutes each and excess buffer solution was tapped off.

After covering the sections with peroxide block for 15-20 minutes, they are washed gently with PBS 3-4times for 2 minutes each. The sections were then covered with power block for 15-20mins, after tapping the excess buffer.

Immuno histochemistry was done 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 hr at room temperature and washed gently with PBS 3-4 times for 2 minutes each. After gentle washing with PBS 3-4 times for 2 minutes each, tissue sections were added with super enhancer and are left for 30 minutes. After the excess buffer is tapped off, secondary antibody was incubated on the tissue sections for about 30 minutes and then washed with PBS 3-4 times for 2 minutes each and covered for 10 minutes with substrate chromogen solution which is prepared freshly, followed by gentle washing using distilled water for 2 minutes. Later,

the sections were immersed in Harri’s hematoxylin for 2 minutes and are washed under running tap water for bluing.

Finally dehydrated through series of absolute alcohol, 70% alcohol, 80% alcohol each for 5 minutes. The tissue sections were then immersed in xylene for final clearing. Later, the tissue sections are mounted by using DPX.

Interpretation of results: If end product of brown colour was present at the target antigen site, it is indicative of positive immunoreactivity The cytoplasm and nucleus of cells present in the tissue sections were stained positive by the specific antibodies used.

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, and the dendritic cells (DCs) by counting that were factor XIIIa positive in HM (Figure 3) and HPIM (Figure 4). Counts of cells of CD1a+ and factor XIIIa+ 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.

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

Results: 15 subjects, 6 Males and 9 Females, with mean age of 32. 46 years were included in the study. (Table-1and Graph-1). 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-2 and Graph-2) The number of Factor XIII a (Dendritic cells) in epithelium and lamina propria in Group I and Group II were 30. 37 ± 5. 42 and 86. 93 ± 13. 99 and 50. 47 ± 7, 27 and 124. 33 ±10. 27 respectively. Statistically significant difference in the number of cells in epithelium and Lamina propria of Group I and Group II was noted. (P= 0001 and P= 0. 001) (Table-3and Graph-3)

Discussion: Periodontitis is an infection-induced inflammatory disease characterized by loss of periodontal connective tissue and the underlying alveolar bone. DCs are phagocytic cells that are similar to the dendrites of nerve cells exhibiting long finger-like processes. DCs reside in an immature state with an higher level of phagocytic capacity, and have an ability to act as sentinels that survey the tissue mainly to invade microbes.

Upon infection, DCs target these invaders and initiate a maturation process via various mechanisms followed by an expression of chemokine receptor 7 (CCR7) that mediates their migration to the lymph node , and up-regulation of MHC class II and co stimulatory molecules, which enable potent activation

of CD4+ T cells. The term professional APCs was awarded to DCs because of this capacity to present antigen via MHC class II (in addition to presentation by MHC class I to CD8+ T cells). Hence, DCs are the most potent APCs activating native T cells, indicating their critical function in induction of adaptive immunity.

Similar to the skin epidermis, the oral epithelium consists of a unique subset of DCs, that is Langerhans cells (LCs) which express 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/CD207.[9] Higher numbers of LCs are found in the sulcular epithelium, whereas LCs are rarely found in the junctional epithelium.

According to Mizumoto et al. LCs express CD1a molecules at remarkably higher levels with almost no detectable CD1b and only modest CD1c expression.[10] So, in this present study CD1a marker was used because of its effectiveness in identification of LCs being a specific marker for LCs. Present study showed that HPIM exhibited a lower number of CD1a+ LCs but a higher number of factor XIII+ a DCs compared to that of the HM in the lamina propria. This difference may explain the different immune responses between gingival and peri implant tissues as seen in a similar study conducted by Marchetti et al.

The present study, when lamina propria of HPIM and HM were compared, significant fewer CD1a+ LCs were found in HPIM when compared to HM. This difference seem to be maintained even after disease establishment, since a reduced number of CD1a LCs was also described for peri-implantitis relative

to the periodontitis. This results in a 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. So, increased proportions of neutrophil granulocytes and macrophages have been observed for peri implantitis comparative to periodontitis.

Titanium may play an important role in the lowering the number of LCs. In this study after titanium exposure, the levels of a number of DCs molecules have been shown to decrease which were similar to that of the study conducted by Chan et al.

Geijtenbeek et al. concluded that DC-SIGN–ICAM-2 and DCSIGN–ICAM-3 interaction regulates chemokine-induced transmigration of DCs across both resting and activated endothelium. Therefore, the essential role in unusual trafficking capacity of DCs is DC-SIGN, which is in addition supported by the expression of DC-SIGN on precursors in blood and on immature and mature DCs, [13, 14] similar to the results of present study.

In the present study, higher number of IDCs were observed HPIM compared to that of the HM. This result may be related with the increased matrix remodeling of peri-implant tissue during the healing process after placement of implant.

Factor XIII a+ dendritic cells are also strong cytokine producers, promoting the increase of TNFα and of IL-8 expression which inturn stimulate type I collagen degradation by gingival fibroblasts. Thus, the reduced number of factor XIIIa+ cells seen in the immunosuppressed subjects who had drug

induced gingival enlargement may result in the reduced secretion of TNF-α, reducing fibroblast proteolytic activity and favour gingival enlargement.

In the lamina propria of the HPIM, a lower number of CD1a+ LCs and a higher number of Factor XIIIa DCs were observed compared to HM. This may be associated with reduced stimulation of the innate and acquired immune responses, a stronger inflammatory reaction, and the more pronounced matrix remodelling seen with peri-implant tissue. Hence, these cells are responsible for more tissue destruction in peri-implant tissue when compared to periodontitis lesions.