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Original Research Article

Evaluation of the distribution of dendritic cells in healthy peri-implant mucosa in comparison to that of healthy gingiva: an original research

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ABSTRACT

Background: Biofilm formation on the implant surface mainly results in peri-implant diseases which are characterized by the inflammatory destruction of the implant supporting tissues. The host response to infection differs between peri-implantitis and periodontitis, but the mechanisms underlying these differences are not clear till date. In the present study, the distribution of dendritic cell sub populations in healthy peri-implant mucosa (HPIM) was compared with healthy mucosa (HM).
Methods: A total of 15 nonsmoker subjects were 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 in the samples. Statistical analysis used Wilcoxon matched paired test was used to compare the distribution of cells in epithelium and lamina propria.
Results: Mean number of factors XIIIa DC in epithelium and lamina propria in group I were 30.37±5.42 and 86.93±13.99 and group II were 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 were noted (p=0.001).
Conclusions: In the lamina propria of HPIM, a higher number of factors XIIIa+ DC were observed compared to HM.

Keywords: Dendritic cells, Peri-implant mucosa, Periodontal tissue, Titanium implants

INTRODUCTION

Immunology has mainly been focused on antigens and lymphocytes, but presence of these two parties does not always lead to immunity. A third party, the dendritic cell (DCs) system of antigen presenting cells (APCs) are the initiators and modulators of the immune response. DCs are efficient stimulators of T and B lymphocytes. B cells, the precursors of antibody-secreting cells, have a capacity that can directly recognize native antigen through their B-cell receptors. T lymphocytes, need the antigen to be processed and presented to them by antigen presenting cells. Intracellular antigens, cut into peptides in the cytosol of the APCs and then tend to bind to major histocompatibility complex (MHC) class I molecules and are recognized by cytotoxic T cells (CTLs), that can directly kill a target cell.[1]

Peri-implant diseases are a group of "contemporary" oral infections which has emerged as a outcome of increased rate of Osseo integrated dental implants in clinical practice. They are mainly characterized by the inflammatory destruction of the supporting tissues around the implant placed, as a result of biofilm formation on the implant surface.[2]

DCs represent a large family of antigen-presenting cells (APCs) that circulate through the bloodstream and are found in nearly all tissues of the body, but their distribution in the peri-implant mucosa is largely unknown. DCs serve as a connection between the innate immune system and the adaptive immune response and are the most potent type of APCs. In their immature state, DCs capture microbes and their antigens; in the mature state, these immune cells stimulate a T- cell response to these captured antigens. Immature DCs, are fewer effective initiators of immunity but are specialized in capturing and processing antigens to form MHC peptide complexes. Thus, two key functions of DCs are they first hold antigens and then, as mature DCs, stimulate T cells.[3] Interstitial dendritic cells (IDCs) are found in gingival tissue. They are observed more frequently in the oral epithelium, than in the sulcular / junctional epithelium, in peri-implantitis, healthy peri-implant tissues, and aggressive periodontitis.[4] The aim of present study was to evaluate the distribution of DCs in healthy peri-implant mucosa (HPIM) and compared to healthy mucosa (HM).

METHODS

The present study is an observational study conducted at department of periodontics, Kaminenei institute of dental sciences, Narketpally, Telangana, India over a period of 6 months i.e., February 2016 - August 2016. 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, embedded in paraffin wax and were sectioned to the thickness of 3 µm with rotary microtome. All the sections were taken onto super frost glass slides, and then placed in the hot air oven at 100ºC for 10 mins for the process of deparaffinisation and were rehydrated by taking through two changes of xylene, absolute alcohol, 70% alcohol, and 80% for 5 minutes each. The slides were placed under running tap water for 2 to 5 minutes.

Antigen retrieval

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

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

Immuno-histochemistry was used to quantify the number of 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

Indicative of positive immunoreactivity was presence of brown coloured 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.

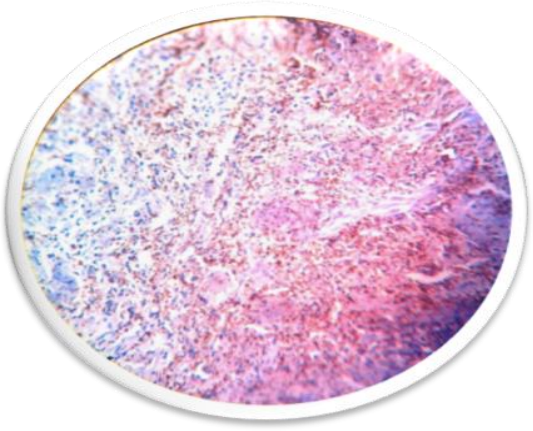

Figure 1: Immuno-histological picture of factor XIIIa DCs in healthy mucosa.

Histomorphometric quantification procedure was performed for and the dendritic cells (DCs) by counting the cells that were factor XIIIa positive in HM (Figure 1)

and HPIM (Figure 2). The cells were identified based on nucleic and cytoplasmic staining and their dendritic shape. Counts of cells of 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.

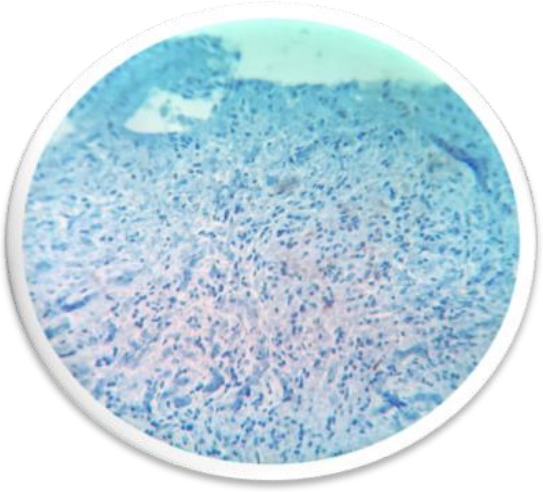

Figure 2: Immuno-histological picture of factor XIIIa DCs in healthy peri-implant mucosa

The cells were counted in 5 fields, which were randomly selected under 100x magnifications. i.e. epithelium and lamina propria of each slide.

Statistical analysis

Wilcoxon matched paired test was used to compare the distribution of factor XIIIa DC in both epithelium and lamina propria in group I and group II.

RESULTS

The present randomized clinical control study is conducted to evaluate the distribution of dendritic cells in peri-implant mucosa an HM. A total of 15 subjects fulfilling the inclusion criteria were selected for the study. Two samples were obtained from each subject. The first sample of HM was obtained prior to the implant placement (group I) and the second sample of peri implant mucosa was obtained at the time of implant exposure prior to placement of gingival formers (group II). On comparison of group I and group II with respect to age, the mean age of subjects enrolled in the study was 32.46 years. Distribution of male and females in group I and group II were 6 males and 9 females in the study. 15 subjects, 6 males and 9 females, with mean age of 32.46 years were included in the study. The number of factors XIII a DC in epithelium and lamina propria in group I were 30.37±5.42 and 86.93±13.99 and in and group II were 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 were noted (p=0001) (Table 1, Figure 3).

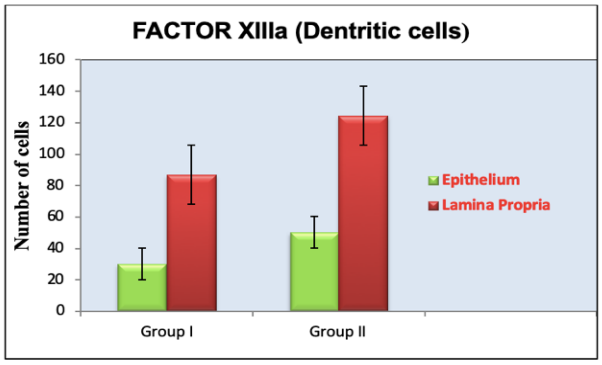

Figure 3: Comparison of distribution of factor XIIIa DCs in epithelium and lamina Propria in group I and group II.

Table 1: Comparison of distribution of factor XIIIa DCs in epithelium and lamina propria in group I and group II by Wilcoxon matched pairs test.

Variable	Epithelium	Lamina propria	P
Group I	30.37±5.42	86.93±13.99	0.001*
Group II	50.47±7.27	124.33±10.27	0.001*
Group I vs Group II	p=0.001*	p=0.001*	

P≤0.05* is significant (Wilcoxon Signed Rank Test).

DISCUSSION

Periodontal diseases are the most common bone diseases in human, having significant economic and medical consequences.[5] Moreover, periodontal diseases are considered as a risk factor for life-threatening conditions such as cardiovascular disease, diabetes, and chronic obstructive pulmonary disease.[6] Although the presence of periodontal pathogens is required for disease initiation, they are not sufficient to cause periodontitis. It has been established that host immune response plays a central role in the destruction of periodontal tissues.[7] Periodontitis is an infection-induced inflammatory disease characterized by loss of periodontal connective tissue and the underlying alveolar bone.[8]

The accumulation of bacterial plaque in the periodontal environment is rapidly monitored by the innate immune system, the first line of defense against invading pathogens.[9] The innate immune system includes anatomical barriers, secretory molecules, and cellular elements such as neutrophils, macrophages, and DCs. These cells are equipped with various receptors enabling them to identify various oral invaders via recognition of pathogen-associated molecular patterns for instance lipopolysaccharide (LPS) or flagellin.[10] Toll-like receptors (TLRs) represent an important family of such receptors with the capability to bind several bacterial and viral molecules.[11] Following receptor triggering, the cells are activated and act in concert to induce local inflammation that eliminates the pathogen and repairs

tissue damage.[12] Because the activity of innate immune cells is limited in its specificity to the pathogen, the development of a highly specific adaptive immunity takes place in parallel.[13] The adaptive immune cells, represented by T and B lymphocytes, infiltrate the infected oral tissue few days later and efficiently mediate pathogen clearance while providing long-lasting immunological memory.[14,15]

DCs are phagocytic cells that are similar to the dendrites of nerve cells exhibiting long finger-like processes.[16] They are a heterogeneous population that is widely distributed in lymphoid and nonlymphoid tissues.[17] In the periphery, DCs reside in an immature state with high phagocytic capacity, contributing to their ability to serve as sentinels that survey the tissue for invading microbes. Upon infection, DCs target these invaders via various mechanisms and initiate a maturation process. The latter involves expression of chemokine receptor 7 (CCR7) that mediates their migration to the lymph node (LN), and up-regulation of MHC class II and co-stimulatory molecules, which enables potent activation of CD4+ T cells. This capacity to present antigen via MHC class II (in addition to presentation by MHC class I to CD8+ T cells) awarded DCs with the term professional antigen-presenting cells (APCs). In fact, DCs are the most potent APCs activating native T cells, demonstrating their critical function in induction of adaptive immunity.[18]

Depending on the conditions, DCs can stimulate the outgrowth and activation of a variety of T cells, which affect the immune response differently. They can persuade CTLs, which express the accessory molecule CD8 and hence interact with MHC class I bearing cells, to proliferate vigorously, which is unusual for CD8+ T cells.[19,20] CD4-expressing T-helper cells, on the other hand, scrutinize cells that express MHC class II molecules. In the presence of mature DCs and of the IL-12 they produce, these T cells turn into interferon-g (IFN-g)-producing Th1 cells. IFN-g activates the antimicrobial activities of macrophages and, together with IL-12, it promotes the differentiation of T cells into killer cells.[60] The capacity of DCs to produce IL-12 and Th1 cells will lead to microbial resistance. With IL-4, however, DCs induce T cells to differentiate into Th2 cells which secrete IL-5 and IL-4. These cytokines activate eosinophils and help B cells to make the appropriate antibodies, respectively.[16] Thus the communication between DCs and T cells seems to be a dialogue rather than a monologue in which the DCs respond to T cells as well.[23] According to Dereka et al DC containing the protransglutaminase-clotting enzyme factor XIIIa represent a specific subpopulation of dermal Dendritic cells that are found in close association with blood vessels. So, In the present study factor XIIIa marker was used for identification of DC.[24]

Present study showed that HPIM exhibited a higher number of factors 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.[25] The difference seemed to be maintained even after disease establishment. 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 implant it is comparative to periodontitis.

This 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.[27] Although titanium has a high resistance to corrosion and exhibits excellent biocompatibility, biocorrosion has been detected in the surrounding tissue when implants had been present for a significant period of time and titanium ions have been shown to regulate DC molecules. And after titanium exposure, the levels of a number of DC molecules have been shown to decrease. Hence this study is also in accordance with Chan et al.[28]

Factor XIIIa+ cells represent interstitial DCs that lack Birbeck granules and Langerin but express DC-SIGN, a lectin-like C-type receptor that binds intercellular adhesion molecule (ICAM)-3 and ICAM-2 on lymphoid T-cells for the adhesion of naive T-lymphocytes on DCs.

This study is also in accordance with the study conducted by Geijtenbeek et al.[29,30] in which he concluded that the DC-SIGN–ICAM-2 and DC-SIGN–ICAM-3 interaction regulates chemokine-induced transmigration of DCs across both resting and activated endothelium. Thus, DC-SIGN is central to the unusual trafficking capacity of DCs, further supported by the expression of DC-SIGN on precursors in blood and on immature and mature DCs in both peripheral and lymphoid tissues. More IDCs were observed in the lamina propria of the HPIM compared to that of the HM similar to the present study. This result may be associated with the increased matrix remodeling of peri-implant tissue during the healing process after implant placement. Factor XIIIa DC stimulate type 1 collagen degradation by gingival fibroblasts.[31]

A reduced number of IDCs in immunosuppressive drug-induced gingival enlargement has been shown, where a lowering of fibroblast proteolytic activity favors gingival enlargement.[32]

Factor XIIIa+ Dendritic cells are strong cytokine producers, promoting the increase of tumor necrosis factor alpha (TNF-α) and of IL-8 expression which stimulate type I collagen degradation by gingival fibroblasts. Thus, the reduced number of factors XIIIa+ cells seen in the immunosuppressed subjects who had DIGE may result in the reduced secretion of TNF-α,

lowering fibroblast proteolytic activity and favoring gingival enlargement.[33]

Considering the limitations of the study such as the small sample size and a short duration of time there is a need for further extensive studies to understand in detail about many features of DCs to allow successful manipulation of the immune system.

CONCLUSION

In the lamina propria of the HPIM, 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 remodeling seen with peri-implant tissue. Hence, these cells are responsible for more tissue destruction in peri-implant tissue when compared to periodontitis lesions.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee, Kamineni Institute of Dental Sciences, Narketpally, Telangana (KIDS/IEC/ 2013/28)

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