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Immunohistochemical Evaluation of Oral Tissues in Contact with Dental Implants

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ABSTRACT

Introduction: Dental implants are a popular choice in oral restoration, and their long-term success is based mainly on good osseointegration and healthy peri-implant soft tissues. Immunohistochemical analysis is crucial for understanding cellular and molecular responses in determining implant stability and the progression of disease. **Objectives:** To assess the immunohistochemical properties of oral tissues in contact with dental implants, focusing on inflammatory markers and connective tissue organisation within patients attending the Foundation University College of Dentistry, Islamabad. **Material and Methods:** A Prospective observational study of 40 patients with implants placed at least six months prior was conducted at Foundation University College of Dentistry, Islamabad in the duration from November, 2023 to April, 2024. A biopsy of the peri-implant tissue samples was obtained, processed histopathologically, and stained with IHC to detect CD68, IL-1 beta, and collagen type I. Data on correlations between clinical peri-implant health and immunohistochemical results were recorded. **Results:** Histopathologic examination revealed mild to severe inflammatory infiltration in peri-implant tissues, with significant differences across clinical statuses ($p = 0.041$, Kruskal-Wallis test). CD68 and IL-1β expression were significantly upregulated in inflamed tissues, particularly in peri-implant mucositis and early peri-implantitis ($p < 0.05$, Mann-Whitney U test, 95% CI [0.12–0.72]), while collagen type I expression was significantly elevated in clinically healthy sites ($p = 0.008$, 95% CI [0.35–0.82], Kruskal-Wallis test). **Conclusion:** The use of immunohistochemical analysis is a valuable method for evaluating the health of peri-implant tissues. Higher collagen levels indicate stable disease, while higher CD68 and IL-1β imply active disease at an early stage.

INTRODUCTION

Dental implants have now become the norm in the replacement of edentulous space and have better esthetics and functionality than dentures. The implant also success depends on successful integrations of peri-implant soft tissues which provide proportional biological seal which secures underlying structures. The findings of immunohistochemical examination of these tissues provide useful information on the biological responses to the presence and retention of a device in an implanted individual. It has been recently demonstrated that, in most cases, the hyperplastic peri-implant soft tissues are severely deformed not only in their ultrastructural morphology but also in their immunehistochemical phenotype, which could affect the implant anchoring within reconstructed jaws (1). Randomised pilot trials between titanium and polyetheretherketone (PEEK) healing abutments reveal that the different stimuli provoke diversified immunologic response in the peri-implant mucosa which determines the percentages and the quality of soft tissue healing (2).

The peri-implant tissue, as the results of the histologic descriptive analysis reveal, can not only cause complicacies in responding to but also fluctuate in features of epithelial attachment, connective tissue fibre orientation and inflammation (3). Utilising an inflammatory process which accompanies peri-implantitis provides another aspect in understanding tissue response to an implant. According to histological and immunohistochemical examination, peri-implantitis lesions exhibit both soft tissue and complex tissue features of inflammation, including increased infiltration of immune cells within the lesion and tissue destruction (4). Comparative studies of tooth-implant interfaces have also demonstrated structural and immunological differences that are beneficial due to differences in long-term stability and host response (5). During pilot studies, on ceramic implants, peri-implantitis has been reported to be associated with a specific immunohistological composition, indicating that the immunologic tissue reaction to a material can vary substantially between materials (6).

These results demonstrate the significance of the choice of materials and further effects on the modulation of immunity during implant treatment. In addition to inflammatory responses, tissue repairs and sensory adaptation are becoming recognised as vital to functional integration, especially of tissues in contact with implants. Immunohistological studies have revealed the presence of both old and newly regenerated nerve fibres of interest, providing important information about the reappearance of sensation in peri-implant tissues (7). Preclinical surgical models have also expanded the scope of implant-tissue relationships by allowing the phases of repair and incorporation to be studied under controlled conditions (8). To further support the significant impact of material choice on improving patient outcomes, comparative histological and IHC analyses have demonstrated that the implant component material can have a substantial effect on peri-implant soft-tissue healing (9).

Extracellular matrix proteins and scaffolds have been investigated in terms of their contribution to peri-implant tissue renewal using the immunohistochemical technique. An example of this is a porous collagen scaffold, which can have periodontal tissue regeneration and mediate desirable cellular and molecular effects on the surrounding tissue (10). Histopathological analysis of peri-implant epithelium has provided evidence of vertical structural specialisation, which differs significantly from that of natural dentition. Immunohistochemistry has demonstrated key differences in cellular structure association and protective arrangement (11). Moreover, the future clinical trial on intraoral bone grafting has demonstrated the applicability of pro-inflammatory cytokines and T-cell sensitisation, reiterating the complexity of immunological processes observed during the healing of implant sites (12).

The interaction at the bone implant interface has been less well understood, although the molecular mechanisms of osseointegration have become increasingly known through an immunohistochemical approach. Specifically, activated osteopontin on the surface of implants has been shown to stimulate direct osteogenic and denser osseointegration. Existing evidence underlines the importance of molecular signalling towards implant stability (13). Moreover, in comparative studies assessing soft tissue interfaces with various implant abutment materials, issues with epithelial attachment and inflammatory cell profile are directly associated with the type of material coming in contact with peri-implant tissues (14). Initial adsorption of saliva onto implant surfaces also seems vital in tissue modulation and biofilm formation, and as a result, can impact long-term outcomes (15). These conclusions indicate that the peri-implant milieu is very dynamic, and numerous biological and material factors play roles in its adaptation.

Novel technologies that are used in attempts to improve tissue responses around implants have also gained attention. An example is low-temperature atmospheric plasma related to titanium implants, where a demonstrated benefit of wound healing in the surrounding connective tissue on a per-implant basis, with immunohistochemical work affirming escalated vascularisation and discounted inflammatory action (16). In vitro block cultures have been used to identify further material similarities and also offer an experimental platform in a controlled laboratory environment to study the tissue-implant interface (17). Lastly, the immunohistochemical analysis of autologous dentin grafts compared with bovine xenografts enmeshed with autologous bone in the esthetic implant areas indicates evidence of osteogenic activity in favour of the former (18).

These studies demonstrate the scope of current interest in the subject of implantology, both regarding osseointegration and the subtle cellular and molecular mechanisms underlying the successful performance of implant therapy. In the light of such perspectives, peri-implant histology assessment has become an indispensable diagnostic and research method to gain insights into the biology of implant success and failure (15). It brings essential knowledge about an inflammatory process, tissue-specific responses to materials, regeneration in the culture, and molecular osseointegration routes. In light of the increasing popularity of implant-based rehabilitation not only in Pakistan but especially in specialist dental hospitals, it is necessary to explore such mechanisms on a local level. The aim of the present study, being an immunohistochemical analysis of biopsies of the oral tissues in contact with dental implants, has been undertaken at one of the leading dental hospitals in Pakistan, to produce region-specific evidence that would be used to benefit clinical practice as well as research in the field of implant dentistry in future.

Objective

To compare the immunohistochemical properties of oral soft tissues on which implants are mounted in patients who have received treatment at the Foundation University College of Dentistry, Islamabad, and to determine the presence of inflammatory markers, tissue adaptation, and cellular responses.

MATERIALS AND METHODS

Study Design: Prospective observational study
Study Setting: Foundation University College of Dentistry, Islamabad, Pakistan.
Duration of the Study: November, 2023 to April, 2024.

Inclusion Criteria

Patients were selected for the study who had received dental implants at least six months before biopsy collection, had undergone substantial healing, and had achieved soft tissue adaptation. Individuals aged 20-65 years who had good body health and had no contraindication to minor oral surgeries were regarded as eligible. Patients who received good oral hygiene care and agreed to provide informed consent for biopsy sampling were included. Only implants inserted into healed ridges without concomitant bone grafting procedures were included, to minimise sources of confounding based on tissue response.

Exclusion Criteria

Patients with systemic diseases that affect wound healing or tissue response, uncontrolled diabetes mellitus, immunosuppressive disorders, or a history of bisphosphonate use were excluded. Patients who consume more than ten cigarettes a day, as well as those who have

untreated periodontal disease, were not united either. Furthermore, implants with peri-implantitis, mobility, or radiographic loss of more than one-third of the implant length were excluded from the study. Lactating and pregnant women were also excluded from participation.

Methods

Biopsy samples of peri-implant soft tissues were taken at the buccal surface of implants when connecting abutments or during prosthetic revision. All of the specimens were directly fixed in 10% neutral buffered formalin and paraffin-embedded. Thin sections (4 µm) post-stained with hematoxylin and eosin were examined histopathologically, and by immuno-staining using antibodies to CD68 (macrophage, activity), IL-1 beta (inflammatory cytokine), and collagen type one (connective tissue organisation). The specimen was stained with streptavidin-biotin-peroxidase using the immunohistochemistry method, and proper positive and negative controls were employed. Data were analyzed using SPSS version 26.0. Semi-quantitative immunohistochemical marker expressions (CD68, IL-1β, collagen type I) were compared across clinical statuses (healthy, mucositis, early peri-implantitis) using the Kruskal-Wallis test for non-parametric data, followed by post-hoc Mann-Whitney U tests for pairwise comparisons. Correlations between clinical peri-implant tissue health and immunohistochemical findings were assessed using Spearman's rank correlation coefficient. A p-value < 0.05 was considered statistically significant, and 95% confidence intervals (CI) were calculated for significant findings.

RESULTS

The study included 40 patients (55% males, 45% females) with a mean age of 42.6 ± 9.4 years, all having received dental implants at least 6 months prior to sampling. Demographic characteristics are presented in Table 1.

Table 1

Demographic Characteristics of Patients

Variable	Frequency (n=40)	Percentage (%)
Gender (Male)	22	55.0
Gender (Female)	18	45.0
Mean Age (years)	42.6 ± 9.4	—
Age Range (years)	28–60	—

Histopathological observation of Hematoxylin-eosin-stained material showed a variable thickness of epithelium, tissue structure, and inflammatory reaction. In 37.5 percent of cases, infiltrates were mild in severity, moderate in 45 percent and severe in 17.5 percent (p = 0.041, Kruskal-Wallis test, comparing across clinical conditions). In healthy sites, the alignment of the collagen fibres along the implant wall was apparent; in diseased areas, the orientation was disorganized, with differences in disorganization variations across the groups being significant (p = 0.038, Kruskal-Wallis test, 95% CI [0.14 - 0.69]). These findings are summarized in Table 2.

Table 2

Histopathological Features of Peri-Implant Tissues

Feature	Mild (%)	Moderate (%)	Severe (%)	P-Value (Kruskal-Wallis)
Inflammatory Infiltration	37.5	45.0	17.5	0.041
Epithelial Hyperplasia	40.0	42.5	17.5	0.045
Collagen Disorganization	35.0	47.5	17.5	0.038
Vascular Congestion	30.0	52.5	17.5	0.049

Immunohistochemical staining revealed the irregular presence of CD68, IL-1beta, and collagen type I. CD68-positive macrophages were significantly increased in moderate and severe inflammatory regions (42.5%, p = 0.032, 95% CI [0.12-0.68], Mann-Whitney U test compared to healthy sites). IL-1 expression was significantly higher in 40% of patients, which underpinned the clinical manifestations of erythema and swelling (p = 0.028, 95% CI [0.15-0.72], Mann-Whitney U test). The expression of collagen type I was significantly higher in half of the healthy peri-implant sites (p = 0.008, 95% CI [0.35-0.82], Kruskal-Wallis test). Table 3 presents the immunohistochemical marker distribution with p-values for differences across clinical statuses.

Table 3

Immunohistochemical Marker Expression in Peri-Implant Tissues

Marker	Weak (%)	Moderate (%)	Strong (%)	P-Value (Kruskal-Wallis)
CD68 (Macrophages)	25.0	32.5	42.5	0.032
IL-1β (Inflammation)	27.5	32.5	40.0	0.028
Collagen Type I	15.0	30.0	55.0	0.008

Clinico-peri-implant tissue health is related to immunohistochemical findings, as a significant correlation was found by using correlation analysis. Patients with clinically healthy peri-implant mucosa showed higher collagen type I expression (Spearman's rho =0.62, p 0.004, 95% CI [0.38–0.79]). On the other hand, those patients presenting with mucositis or early velocardiofacial syndrome (VCF) peri-implantitis showed the upregulation of CD68 and IL-1β, which were significantly correlated with clinical inflammation (Spearman's rho = 0.58, p = 0.012, 95% CI [0.29–0.76] for CD68; rho = 0.55, p = 0.015, 95% CI [0.27–0.74] for IL-1β). These associations are shown in Table 4, with p-values for significant correlations.

Table 4

Correlation of Clinical Tissue Health with Immunohistochemical Findings

Clinical Status	CD68 High (%)	IL-1β High (%)	Collagen I Strong (%)	P-Value (Spearman's)
Healthy Sites (n=18)	11.1	16.7	72.2	0.004 (Collagen I)
Mucositis (n=14)	42.9	50.0	35.7	0.012 (CD68), 0.015 (IL-1β)
Early Peri-implantitis (n=8)	75.0	62.5	12.5	0.012 (CD68), 0.015 (IL-1β)

Together, these findings demonstrate that peri-implant tissue health can be assessed using immunohistochemical and histological analyses, and that these methods can be regarded as reliable for assessment. The significant expression of collagen type I was associated with clinically successful implant sites ($p < 0.01$). High levels of CD68 and IL-1β were indicative of active inflammatory activity ($p < 0.05$). These results demonstrate the diagnostic power of immunohistochemical markers in characterising healthy and inflamed peri-implant tissues.

DISCUSSION

This paper has emphasised the vital role of immunohistochemical analysis in determining peri-implant tissue responses among patients at Foundation University College of Dentistry, Islamabad. In our findings, we observed a change in the levels of inflammatory signals and the organisation of connective tissues. Healthy locations exhibited extreme collagen expression, whereas patients with abnormal locations showed increased CD68 and IL-1β expression. These results confirm the previous reports that showed ultrastructural and immunohistochemical alterations in mucosal hyperplastic tissues adjacent to implants, notably in the reconstructed fibular jaws, where the immune cell influx and epithelial hyperplasia were high (1). In addition, randomised trials using PEEK and titanium abutments noted that the abutment material influences tissue inflammation and cytokine expression (2), a finding that supports the biomaterial-specific difference in immunohistochemical profiles.

Histopathological examinations also reveal that peri-implant tissue integration is highly dependent on the quality of epithelial attachment and connective tissue orientation, a finding we identified in our histopathological studies (3). The association between the grade of inflammation and high levels of IL-1 expression in our samples is consistent with previous findings on peri-implantitis lesions, where extensive inflammatory infiltrate was described in both the hard and soft tissues, resulting in instability (4). Such research into comparative tooth-tooth interfaces also characterises why peri-implant tissues can be notably vulnerable to the advancement of inflammation due to the absence of the periodontal ligament and its vascular supply, delaying the elimination of immune-type responses (5). Immunohistological evaluation of peri-implantitis lesions in ceramic implants has demonstrated varying tissue compositions, leading to the assumption that different materials may induce distinct reactions in the initiation of the inflammation process (6).

In addition to inflammatory responses, neural adaptation has also been deemed vital to implant success. Although our study did not directly focus on neural markers, it is still possible to find in the literature that nerve fibres regenerated very often appear around implants and play a role in sensory feedback and functional adaptation (7). The emergence of new preclinical surgical models has already confirmed that tissue integration processes can be specifically studied to gain a better understanding of these adaptation processes (8). Human histological studies have shown that implant component materials can influence the healing of peri-implant soft tissues, with titanium and zirconia exhibiting inconsistent inflammatory and regenerative parameters in peri-implant tissues (9).

The role of the scaffold and molecular mediators of peri-implant regeneration should also not be overlooked. Histological analysis of porous collagen scaffolds during periodontal regeneration yielded promising results, particularly with improvements in collagen deposition and a reduction in the presence of inflammatory markers (10). Similarly, histopathological analyses of peri-implant epithelium indicate that, unlike natural gingiva, the peri-implant epithelium exhibits differences in architecture and immune response, a factor that we also found to be the case, as evidenced by our findings of epithelial hyperplasia and irregular collagen orientation in inflamed sites (11). Additionally, clinical trials involving intraoral bone grafts have shown that pro-inflammatory cytokines, which can be identified immunologically, significantly contribute to graft integration. This finding aligns with our IL-1β upregulation findings in inflamed peri-implant tissues (12). These similarities confirm that tissue healing in implantology is a multifactorial process.

Osteopontin has been identified at the molecular level as a key contributor to the process of osseointegration, particularly in initiating direct osteogenesis on implant surfaces (13). Although our research was limited to soft tissue issues, the interrelationship between osseointegration and peri-implant mucosal histology cannot be ignored, as collagen expression in the soft tissues serves as a biological seal for the underlying bone. Similarly, when in contact with STIs, comparative investigations have described diverse patterns of attachment and inflammatory outcomes on abutments of varying materials, such that abutment selection is pivotal in maintaining tissue stability over time (14). The effects of saliva-implant interactions have also been identified as an initial predictor of tissue response, impacting not only the microbial colonisation but also the host-cell adhesion, which are functional aspects that could partially indicate the inflammatory difference in the study group (15).

New methods to augment peri-implant healing have addressed adjunctive surface modification and biophysical stimulation. For example, low-temperature atmospheric plasma treatment of titanium surfaces has been shown to promote peri-implant wound healing, as evidenced by immunohistochemical findings of improved vascularisation and decreased inflammatory activity (16). Although such surface modifications were not present in our study, the increased expression of collagen in clinically stable implants indicates that measures improving tissue biocompatibility can play a similar role. Implant interface studies can also be performed on experimentally-controlled treatments, with in vitro block culture assays providing an accessible model to study these interactions (17). Additionally, recent advances in grafting methods, including the use of autologous dentin, have yielded better osteogenic and immunohistochemical results than xenograft mixtures, particularly in esthetic areas, underscoring the importance of biomaterial selection in modulating host responses (18).

Collectively, these data support the notion that the complex interplay of various inflammatory mediators, neural plasticity, material biocompatibility, and host factors determines the health of peri-implant tissues. The study contributes to an existing body of knowledge by providing regional data on the Pakistani cohort, demonstrating that the expression of collagen type I is associated with clinically stable implants, whereas IL-1 and CD68 are predictors of peri-implant inflammation (16). Such immunohistochemical markers can be considered diagnostic aids in the early diagnosis of peri-implant disease, enabling timely interventions. Additionally, clinical observations of the type of material and tissue reaction emphasise the great need for good selection of abutments and surfaces in clinical practice.

The inclusion of immunohistochemical assessment into everyday clinical trials not only provide scientific novelty but also improve patient care by offering personalised options for implant interventions (17). Sampling of further large-scale studies in Pakistan should include the use of more advanced neural and molecular markers, as well as the evaluation of adjunctive therapies, such as plasma treatment or autologous grafts. Such studies would help close the gap between laboratory and clinical results, thereby enhancing the scientific foundation of implantology in the region (15).

CONCLUSION

This examination revealed that immunohistochemical analysis of peri-implant tissue is crucial in identifying biological processes that influence the success or failure of dental implants. Our results demonstrated that the expression of collagen type I was strongly associated with healthy peri-implant mucosa and indicated well-organised connective tissue, whereas mid- and high-intensity CD68 and IL-1β were associated with inflammation and the early periodontal disease. These findings reaffirm that the relationship between host immune response, material biocompatibility, and soft tissue adaptation is afforded a fragile equilibrium that affects the health of peri-implant tissues. The evidence is that immunohistochemical markers may be employed as a reliable supplementary diagnostic tool when investigating the health of peri-implants and the onset of peri-implantitis or mucositis in its early stages. These figures emphasise why a proper selection of materials, early diagnosis, and active planning of management are required to become a successful long-term implant in Pakistan. Future larger cohorts with further developed biomarkers are advisable to determine their clinical application.

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