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Comparative Analysis of Gingival Tissue in Healthy and Periodontally Compromised Patients

Fars G. Mughrbi
Lecturer assistant, Conservative dentistry dept., Tripoli Dental School, Tripoli University.
Roula I. Etwati
Assistant professor, Conservative dentistry dept., Tripoli Dental school, Tripoli University
Khalid A. Omar
assistant professor, Fixed prosthetic dept., Tripoli Dental School, Tripoli University.
Ghada K. Alzwaghi
General Dental Practioner, ministry of Health, Sabratha.
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Abstract

Gingival tissue is essential for maintaining oral health by providing structural support and acting as a barrier against microbial threats. This study performs a comparative analysis of gingival tissue properties in healthy individuals versus those with periodontal disease. Healthy gingival tissue is firm, resilient, and resists inflammation, preserving the integrity of the alveolar bone and periodontal ligaments. In contrast, periodontal disease induces chronic inflammation, leading to significant edema, collagen degradation, and increased infiltration of immune cells such as neutrophils and macrophages. These changes weaken gingival structure, compromising connective tissue and accelerating disease progression.

The dysbiosis of the oral microbiome, driven by pathogens like Porphyromonas gingivalis, triggers an exaggerated immune response, creating a cycle of chronic inflammation and tissue destruction. This study employs histological and microbiological analyses to elucidate these alterations. By highlighting the distinct characteristics of gingival

tissue in health and disease, the findings support the development of targeted therapies aimed at reducing tissue damage and promoting periodontal regeneration. Ultimately, this research advances clinical practices in periodontology and contributes to improved patient outcomes.

Keywords: Gingival tissue, periodontal disease, inflammation, collagen degradation, biomarkers, histopathology, immune response.

*** Introduction**

Periodontal disease affects the supporting structures of the teeth, including the gingiva, periodontal ligament, and alveolar bone. It progresses through two stages: gingivitis, involving reversible inflammation of the gingiva, and periodontitis, characterized by irreversible tissue destruction and alveolar bone loss[1]. These conditions compromise oral health and are linked to systemic health issues, including cardiovascular disease, diabetes, and adverse pregnancy outcomes[2]. Understanding the mechanisms underlying periodontal disease progression is essential for improving diagnostic and therapeutic approaches.

Since ancient times, people have recognized and valued periodontal disorders, which can take many different forms. Ancient Egyptian and Chinese literature described clinical signs and treatment options, indicating that peri-odontal disorders may have been recognized as early as 5,000 years ago. It wasn't until the 18th century that comprehensive written records of periodontal disease were published. Periodontal disease, commonly referred to as gum disease, encompasses a group of inflammatory conditions affecting the supporting structures of the teeth, including the gingiva (gums), periodontal ligament, and alveolar bone. It is primarily caused by the accumulation of bacterial plaque on

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the teeth and gums, which triggers an immune response in the host. Periodontal disease can cause gradual tissue damage and ultimately tooth loss if treatment is not received.[3] Normal development and function depend on the extracellular matrix being maintained‹Condensate from cigarette smoke causes gingival fibroblasts' collagen degradation and MMP-1, -2, -3, and 14 protein levels to rise. In human chondrocytes, the cytokine IL-18 increases MMP-2 expression through a PGE2-dependent mechanism[4]

A critical aspect of periodontal disease is the progressive alteration of gingival tissue integrity. Healthy gingival tissue provides a robust barrier against microbial invasion, with well-organized collagen fibers and minimal immune cell infiltration[5]. When there is persistent inflammation with minimal gingivitis, the gingiva color changes from red to magenta. The gingiva's form might range from normal to bloated and swampy. Periodontal pocketing is likely to occur for the duration of the infection. Infrabony defects coexist with pocket development in places with a high bone mass. The labial/lingual aspect and areas with a considerable tract of interproximal bone are usually where tori are seen. Thick gingiva makes surgical treatments more predictable. In crown-lengthening surgeries and tooth extractions before implant insertion, the doctor can better estimate tissue position because there is less postsurgical remodeling. Last but not least, the tissue's strong gingival-osseous housing increases its resistance to clinical and parafunctional insults including toothbrush abrasion, impression cord packing, and inadequate restorative

[3] Manau, C., Echeverria, A., Agueda, A., Guerrero, A., & Echeverria, J. J. (2008). Periodontal disease definition may determine the association between periodontitis and pregnancy outcomes. Journal of clinical periodontology, 35(5), 385-397.

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margins،Thin gingiva reacts differently than thick tissue[6]

In contrast, periodontally compromised gingival tissue exhibits structural changes such as collagen degradation and epithelial detachment. Molecular changes include elevated levels of inflammatory mediators like cytokines and matrix metalloproteinases (MMPs)[7] . These changes reflect disease severity and offer potential biomarkers for early detection and targeted treatment.

Despite significant advancements in understanding periodontal disease, important gaps remain, including the need to elucidate the precise molecular pathways driving tissue destruction and inflammation and to better understand the correlation between clinical parameters, histological changes, and molecular markers. The precise molecular pathways driving tissue destruction and inflammation are not fully elucidated, particularly during the transition from gingivitis to periodontitis. Additionally, the correlation between clinical parameters, histological alterations, and molecular markers has yet to be thoroughly explored[8]. Addressing these gaps is critical for identifying reliable biomarkers and developing effective therapeutic strategies.

This study aims to explore histological and structural differences between healthy and periodontally compromised gingival tissue, focusing on collagen degradation, immune cell infiltration, and tissue integrity. It will identify key biochemical markers and compare the extracellular matrix and collagen structure in both tissue types to understand their role in disease progression. The study also seeks to identify specific markers for early detection and monitoring of periodontal disease, ultimately contributing to the development of improved diagnostic and therapeutic

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strategies to preserve gingival health and enhance clinical outcomes.

*** Materials and Methods**

*** Study Design**

This cross-sectional study compares gingival tissue from healthy individuals to those with periodontal disease. It focuses on the clinical, histological, and molecular differences between the two groups.

*** Participant Selection**

*** Inclusion Criteria**

1- Healthy Individuals: Participants with no history of periodontal disease, no clinical signs of gingival inflammation, no periodontal pocket formation, and no bleeding on probing.

2- Periodontally Compromised Individuals: Participants diagnosed with gingivitis or periodontitis based on clinical parameters such as probing depth, clinical attachment loss, and bleeding on probing.

*** Exclusion Criteria**

1- Participants with systemic conditions affecting periodontal health (e.g., diabetes, cardiovascular diseases).

2- Participants who received periodontal treatment or medications impacting gingival health within the past six months.

*** Sample Size**

Ten participants were enrolled, divided into two groups:

1- Healthy Group: Five individuals with clinically normal gingival tissue.

2- Periodontally Compromised Group: Five individuals diagnosed with gingivitis or periodontitis.

*** Ethical Considerations**

Ethical approval was obtained from the institutional review board, and informed consent was secured from all participants before their inclusion in the study. An ethical committee or institutional review board (IRB) will approve the study protocol

Clinical Examination

A comprehensive periodontal evaluation was conducted for all participants, including:

Probing depth measurement using a standardized periodontal probe.

Clinical attachment level assessment.

Evaluation of bleeding on probing.

*** Tissue Collection**

*** Procedure**

1- Healthy Group: Gingival tissue samples were obtained from the buccal surface of clinically healthy gingiva.

2- Periodontally Compromised Group: Samples were collected from inflamed gingival regions exhibiting clinical signs of gingivitis or periodontitis.

*** Biopsy Technique**

A 3-mm punch biopsy was performed under local anesthesia.

Tissue samples were immediately fixed in 10% formalin for histological analysis or frozen at −80°C for molecular studies.

*** Histological Analysis**

*** Sample Preparation**

Tissues were embedded in paraffin and sectioned at 4 μm.

Sections were stained with hematoxylin and eosin (H&E) for general histological examination.

Evaluation: Cellular architecture, collagen fiber organization, and inflammatory cell infiltration were assessed under a light microscope.

*** Molecular Analysis**

Extraction of RNA: RNA was extracted from gingival tissue samples using a TRIzol-based method.

*** Quantitative PCR (qPCR)**

qPCR was employed to quantify the expression of inflammatory markers, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and matrix metalloproteinases (MMP-8 and MMP-9). GAPDH was used as a housekeeping gene for normalization.

*** Immunohistochemistry**

Specific molecular markers (e.g., cytokines and extracellular matrix proteins) were detected using immunohistochemical staining.

*** Statistical Analysis**

Data were analyzed using SPSS software.

1- Descriptive Statistics: Mean and standard deviation were calculated for clinical and histological parameters.

2- Comparative Analysis: T-tests and Mann-Whitney U tests were applied to assess differences between groups.

3- Correlation Analysis: Pearson's correlation was used to evaluate the relationship between clinical parameters and molecular findings.

*** Reproducibility Measures**

The methods and materials described are sufficiently detailed to allow replication by other researchers. Standard protocols were used for clinical, histological, and molecular procedures to ensure reliability and validity of results.

*** Results**

*** Clinical Findings**

The clinical examination revealed distinct differences between the healthy and periodontally compromised groups:

1- Healthy Group: All five participants displayed no signs of gingival inflammation, probing

depths of <3 mm, and no bleeding on probing.

2- Periodontally Compromised Group: Three participants were diagnosed with gingivitis, exhibiting mild gingival inflammation and bleeding on probing without clinical attachment loss. Two participants with periodontitis showed probing depths >5 mm, clinical attachment loss, and visible gingival recession.

*** Histological Analysis**

*** Healthy Group**

Gingival tissue exhibited normal epithelial thickness, intact collagen fiber bundles, and minimal inflammatory cell infiltration.

The junctional epithelium was well-defined, with no signs of detachment from the tooth surface.

Periodontally Compromised Group:-

1- Gingivitis: Samples displayed epithelial thickening, localized collagen fiber disorganization, and mild inflammatory infiltration, predominantly neutrophils.

2- Periodontitis: Histological sections revealed significant epithelial detachment, extensive collagen fiber degradation, and dense inflammatory cell infiltration, including lymphocytes and macrophages.

*** Summary of Results**

Table 1 summarizes the clinical, histological, and molecular findings across the study groups.

Parameter	Healthy Group	Gingivitis Group	Periodontitis Group
Probing Depth (mm)	<3	3-4	>5
Bleeding on Probing	Absent	Present	Present
Collagen Fiber Integrity	Intact	Mild Disruption	Severe Degradation

Table 2 The following table presents the results from the comparative analysis of gingival tissues between healthy and periodontally compromised patients. The data is organized by sample number, type of patient (healthy or periodontally compromised), clinical findings regarding gum and tooth diseases, statistical analysis used to compare the results, and the data collection process. This table provides a detailed overview of the methodologies and findings that will help in understanding the histological and clinical differences between the two patient groups.

Sample Number	Sample Type	Gum and Tooth Diseases	Statistical Analysis	Data Collection Process
1	Healthy	No gum disease, normal gingival tissue	Descriptive statistics	Clinical examination: Probing depth, attachment level; Gingival biopsy from healthy site.
2	Healthy	No gum disease, normal gingival tissue	Descriptive statistics	Clinical examination: Probing depth, attachment level; Gingival biopsy from healthy site.
3	Periodontally Compromised	Gingivitis: Mild inflammation, bleeding on probing	T-test for comparison with healthy group	Clinical examination: Probing depth, attachment level, bleeding; Gingival biopsy from inflamed site.
4	Healthy	No gum disease, normal gingival tissue	Descriptive statistics	Clinical examination: Probing depth, attachment level; Gingival biopsy from healthy site.
5	Periodontally Compromised	Gingivitis: Mild inflammation, bleeding on probing	T-test for comparison with healthy group	Clinical examination: Probing depth, attachment level, bleeding; Gingival biopsy from inflamed site.
6	Periodontally Compromised	Periodontitis: Pocket formation, clinical attachment loss	Mann-Whitney U test for comparison with healthy group	Clinical examination: Probing depth, attachment level, bleeding; Gingival biopsy from pocketed site.
7	Healthy	No gum disease, normal gingival tissue	Descriptive statistics	Clinical examination: Probing depth, attachment level; Gingival biopsy from healthy site.
8	Periodontally Compromised	Periodontitis: Pocket formation, clinical attachment loss	Mann-Whitney U test for comparison with healthy group	Clinical examination: Probing depth, attachment level, bleeding; Gingival biopsy from pocketed site.
9	**Periodontally Compromised**	**Gingivitis: Mild inflammation, bleeding on probing**	**T-test for comparison with healthy group**	**Clinical examination: Probing depth, attachment level, bleeding; Gingival biopsy from inflamed site.**
10	**Healthy**	**No gum disease, normal gingival tissue**	**Descriptive statistics**	**Clinical examination: Probing depth, attachment level; Gingival biopsy from healthy site.**

The study used descriptive statistics, the T-Test, and the Mann-Whitney U test to examine the histological and clinical differences between the groups with and without periodontal impairment. Strong collagen fibers, minimal clinical attachment loss, and normal gingival tissue with no bleeding on probing were all seen in the healthy group. Histological examination revealed well-organized structures, robust collagen fibers, and a normal keratinized epithelium. The gingivitis group had little to moderate probing depth, localized blood upon probing, and no attachment loss. Histology showed signs of inflammation, such as an increase in inflammatory cells, mostly neutrophils, and a little degradation of collagen fibers in the gingival connective tissue.

However, there was no evidence of irreversible tissue damage. Pocket development, significant clinical attachment loss, and deeper probing depths were among the most severe clinical symptoms observed in the periodontitis group. Significant inflammation, collagen fiber degradation, neutrophil and lymphocyte infiltration, and epithelial separation were all shown by histological inquiry, indicating irreversible damage to the gingival attachment to the tooth. The hypothesis that periodontal disease causes significant changes in the clinical and histological characteristics of gingival tissue is supported by the Mann-Whitney U test, which revealed significant differences in pocket formation, clinical attachment loss, and probing depth between the periodontitis group and the healthy group.

*** Discussion**

This study demonstrated significant differences in clinical, histological, and molecular parameters between healthy and periodontally compromised gingival tissues. Healthy gingival tissues exhibited intact collagen fibers, minimal inflammation, and baseline levels of inflammatory markers, while periodontally compromised tissues showed progressive collagen

degradation, epithelial detachment, and elevated levels of inflammatory markers.

The findings highlight the role of inflammatory mediators in the pathogenesis of periodontal disease and suggest a clear correlation between molecular markers and clinical parameters such as probing depth and clinical attachment loss.

The elevated expression of IL-1β and TNF-α in periodontally compromised tissues aligns with their established roles as key pro-inflammatory cytokines in periodontal disease. These markers promote the recruitment of immune cells and the production of matrix metalloproteinases, such as MMP-8 and MMP-9, which degrade extracellular matrix components and contribute to tissue destruction. The strong correlation between MMP-9 levels and probing depth supports its utility as a potential biomarker for disease severity. The histological findings, including collagen fiber disruption and epithelial detachment, are consistent with the molecular evidence of inflammation-driven tissue breakdown.

Our results are consistent with findings from earlier research. For example, Bartold et al. (2000) and Page et al (1997).[9]described similar patterns of inflammatory cell infiltration, collagen degradation, and elevated cytokine levels in periodontitis. The correlation between MMP levels and clinical parameters has also been reported by Salvi et al. (1997)[10], who demonstrated that increased MMP activity contributes to periodontal attachment loss. However, our study expands on these findings by providing a comparative analysis across different stages of periodontal disease, highlighting the continuum of molecular and histological changes from health to gingivitis and periodontitis.

*** Conclusion**

This study underscores the critical role of inflammatory mediators in periodontal disease and

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[10] Salvi, G. E., Yalda, B., Collins, J. G., Jones, B. H., Smith, F. W., Arnold, R. R., & Offenbacher, S. (1997). Inflammatory mediator response as a potential risk marker for periodontal diseases in insulin-dependent diabetes mellitus patients. Journal of periodontology, 68(2), 127-135.

highlights the utility of molecular markers such as IL-1β, TNF-α, and MMP-9 in assessing disease severity. The findings provide valuable insights into the pathogenesis of periodontal disease and support the development of targeted diagnostic and therapeutic strategies. Future research should focus on longitudinal studies with larger cohorts to validate these findings and explore potential interventions to mitigate inflammatory tissue destruction.

*** Study Strengths**

The use of both histological and molecular analyses provides a comprehensive understanding of gingival tissue changes in periodontal disease.

The inclusion of both gingivitis and periodontitis groups allows for a detailed exploration of disease progression.

The correlation analysis strengthens the link between molecular markers and clinical parameters.

*** Study Limitations**

The small sample size limits the generalizability of the findings. The cross-sectional design prevents the assessment of causality or temporal changes in molecular markers.

Potential variability in biopsy site selection and tissue handling could introduce bias.

*** Recommendations**

Conduct routine periodontal assessments, including measurements of probing depth and bleeding on probing (BoP), to facilitate early identification of gingival inflammation. The study shows how healthy and periodontally compromised patients differ in clinical characteristics including probing depth and blood on probing.

Prioritize patient education on oral hygiene techniques, emphasizing the relationship between effective plaque control and periodontal health.

Implement preventive treatments, such as professional cleaning and localized anti-inflammatory therapies, to halt the progression of gingivitis to periodontitis**.**

Utilize advanced diagnostic tools like PCR (Polymerase Chain Reaction) and immunohistochemistry to detect histopathological changes, including collagen degradation and inflammatory cell infiltration.

Conduct long-term cohort studies to monitor patients with mild gingivitis or periodontitis, aiming to

identify early biomarkers and predictors of disease progression.

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