ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
June2025 • Vol. 38

Clinical and Histological Evaluation of Gingival Tissues in Different Stages of Periodontitis

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Aim: The objective of the present study was to assess the structure of gingival tissues during various phases of periodontitis and the following were drawn: clinically and histologically

Materials and Methods: In this study thirty-six adult male albino rats weighing between 220-250 grams were employed. The rats were separated into four groups: the control group, which included six rats, group II included ten rats, Group III included ten rats and Group IV included ten rats. Thirty-six male and female patients were chosen among those who visited the department of oral medicine and periodontology's outpatient clinic at al Azhar University's faculty of dental medicine to participate in this study. Patient's ages were between 30 and 60 years. Before beginning any operation, all subjects were getting the information regarding the purpose of the study as well as its advantages. All patients were asked to sign a written consent form indicating that they had been fully informed about the planned research program and experimental design. The samples were prepared for statistical and histological examination

Results: Periodontitis affected gingival health and made Spacing of epithelial cell layers, vacillation of lamina propria, numerous of hyperemic vessels exist in an infiltrated connective tissue. The increasing in inflammatory cells and extravasation of blood vessels were observed.

Conclusion: The gingival health tissue and the severity of periodontitis were directly correlated. In case of the increasing of the gingival inflammatory processes led to the increasing of angiogenesis and inflammatory cell infiltration in the connective tissue and vice versa.

Keywords: Gingival tissues, Periodontitis, Clinical and histological study

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Introduction

A chronic inflammatory illness that is highly common and has multiple causes is periodontitis which leads to the loss of alveolar bone and the destruction of connective tissue connection, which eventually leads to tooth loss.[1]

The typical sign of periodontal disease is the progressive destruction of the periodontal complex's soft and hard tissues. This is controlled by the combination of immune reaction and dys optic bacterial communities in the gingival and periodontal tissues. The local oral microbiota becomes dysbiotic and the inflammation causes tissue damage, leading to an increase in the number of putative periodontal infection. As a result, proteolysis, inflammation, and periodontal pathogen enrichment continue in a positive feedback loop. Periodontal disease progresses as a consequence of ongoing gingival inflammation and associated microbial infections.[2]

The supporting periodontium (alveolar bone, cementum and gingiva tissues) are affected by periodontal disease. It is possible to undergo irreversible changes as a result of persistent activity of the etiological elements that cause and maintain pathological changes. The development of gingival inflammatory processes is linked to angiogenesis and inflammatory infiltration.[3]

This inflammatory infiltration during gingival inflammation contributes to an increase in collagen breakdown or fibrotic reaction inside the connective tissue by the increasing the action of inflammatory mediators of the connective tissue.

From this point, the 1999 Classification was successful in identifying the features of the patients; classes revealed only small variations in the complexity of the disease and risk factors, which influence treatment options and disease prognosis. This is crucial because certain indicators, like furcation involvement and periodontal probing depth (PPD), have been demonstrated to be useful in predicting tooth loss.[4]

Materials and Methods

Sample size calculation

Based on the mean defect depth among the groups under study that was gathered from earlier studies, the sample size was determined. The sample size based on effect size of 2.5153, 2- tailed test, power =90.0 and α error= 0.05 was determined using the G*power program version 3.1.9.4. At least ten rats in each experimental group and six rats in the control group were the suitable sample size.[5]

The experiment was conducted in the animal house, faculty of medicine, Al-Azhar University, Cairo. The animal housing conditions were following the regulations of the animal house as recommended by the faculty's research ethics committee. Rats were housed in individual cages for each group under conventional laboratory conditions, with a temperature of 20 to 25 degrees Celsius, a standard feed, and unrestricted access to water.

Animals

For this investigation, 36 mature male albino rats weighing between 220-250 grams were employed. The experiment was conducted in the animal house, faculty of medicine, Al- Azhar University, Cairo. The animal housing conditions were following the regulations of the animal house as recommended by the faculty's research ethics committee. All experimental protocols were approved by The Research Ethics Committee,

faculty of dental medicine for girls, al Azhar University.

Rats were housed in conventional laboratory conditions in individual cages with three rats per cage, at a temperature between 20 and 25 degrees Celsius. They were also fed a standard food and had unconditional access to water.

Animal groups:

Group I: Control group and included six rats.

Group II: Grade I periodontitis and included ten rats.

Group III: Grade II periodontitis and included ten rats.

Group IV: Grade III periodontitis and included ten rats.

Induction of experimental periodontitis

Under general anesthesia, rats were received intraperitoneal injections of ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight). Experimental periodontitis were induced by placing strile 4/0 silk ligatures in 8 around the mandibular first molar in each group for 14 days. All surgical procedures were carried out under general and local anesthesia causing gingival inflammation and plaque accumulation, and thus the development of periodontal disease were occurred.

Samples Collection

The animals in all groups were euthanized by Anesthetic overdose of thiopental after four weeks, then, all animal's their gingival tissue removed from their mouths and was processed for light microscopic examination and statistically analysis.[6]

With a Light Microscope (L/M)

The specimens were fixed in ten percent neutral-buffer formalin right away, followed by a wash, dehydration, clearing, and paraffin embedding. Then, serial sections with 5µm thicknesses were viewed under a light microscope after being stained with Hematoxylin and Eosin (H&E).[7]

Subjects and Methods

Thirty-six male and female patients were chosen from those working in the periodontics and oral medicine outpatient clinic at al Azhar University's college of dental medicine for girls, to participate in this study.

Patient's ages were between 30 and 60 years. All subjects were told about the purpose and advantages of taking part in this study prior to any procedures. All patients were required to provide written consent that satisfies them regarding the planned research program and experiment design.

The patients were chosen according the following criteria

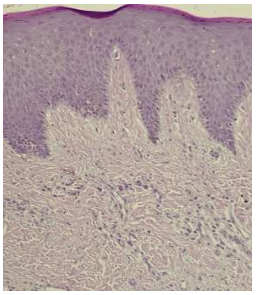
- Stage I to stage III periodontitis according to the criteria of A novel categorization system for periodontal and peri-implant ailments.[8]
- Free from any systemic disease that in accordance with the modified Cornell Medical Index, impact the periodontium or obstruct periodontal therapy.[9]
- No systemic antibiotics, non-steroidal anti-inflammatory drugs, or periodontal therapy during the six months before.
- Not a smoker.
- Not pregnant women's.

Sample size calculation had been done according to the following equation: $4P*Q/d^2$

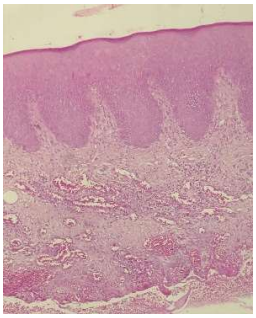
a- P= prevalence f b- Q = 100-P

c- D= allowable error (15% of P) d

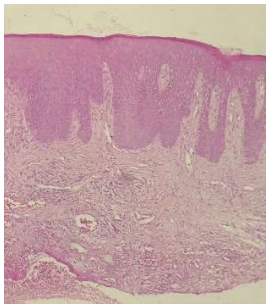
Thirty-six patients were split evenly into the following four groups based on the updated periodontitis classification:



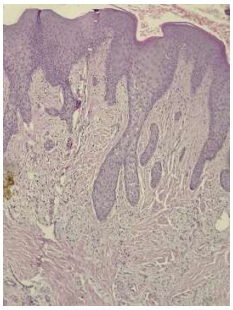
Group I: 9 patients with healthy periodontium.



Group II: 9 patients with periodontitis diagnosed as stage I.



Group III: 9 patients with periodontitis diagnosed as stage II.



Group IV: 9 patients with periodontitis diagnosed as stage III.

Clinical Parameters

The clinical parameters were assessed and documented in compliance with the recently classification parameters.[10]

1- Probing pocket depth (PPD): Six measurements per tooth were made using the University of Michigan 'O' periodontal probe, with Williams's markings.
2- Bleeding when pressed.
3- Clinical attachment loss (CAL): Six measurements per tooth were made using the University of Michigan 'O' periodontal probe, with Williams's markings.

Probing pocket depth (PPD)

We measured the depth of the pocket which started at the free gingival margin and ended at the periodontal pocket's base. William's graduated periodontal probe, which was parallel to the tooth's long axis and graduated 1, 2, 3, 5, 7, 8, 9 and 10 millimeters to the nearest millimeter was used to take the measurements. The Distolingual, Mesiolingual, midlingual, Distobuccal, midbuccal sites were the six sites per tooth that were investigated. For every tooth in each patient, the deepest PPD was chosen.

Gingival index

The patients' gingival condition was evaluated and scored by criteria of Gingival Index according to Löe and Silness.

Thirty six participants participated in the current investigation. Their ages ranged from 20 to 70 years, with 27 males and 9 females. Patients in all were chosen and split up into four groups. Nine patients were in each group. All patients participating in this study had their periodontal health status evaluated using the criteria of Clinical Attachment Level (CAL), PPD and gingival index. Every

evaluated subject's data for the current study was logged, tabulated, and subjected to statistical analysis.

Histological results

Histological examination of the gingival tissue in group I (control group) showed: Stratified squamous keratinized epithelium with normal intracellular spaces, normal thickness of keratin layer and considerable number of macrophage like cells, plasma like cells and lymphocyte like cells in the context of the lamina propria.

Histological analysis of gingival inflammation in group II showed: Stage I periodontitis, spacing of epithelial cell layers, vacillation of lamina propria, numerous of hyperemic vessels exist in connective tissue. Increasing in inflammatory cells and extravasation of blood vessels were also observed in connective tissue.

Histological analysis of gingival inflammation in group III showed: Stage II periodontitis, thinning in keratin layer in some areas and loss in other. Loss of normal architecture of rete pegs of the epithelium, loss of normal arrangement of basal cell layer. Disintegration of the basement membrane appeared. The number of inflammatory cell increased in connective tissue and discontinuity of collagen fibers were also observed.

Histological analysis of gingival inflammation in group IV show: Stage III periodontitis, irregular thickness and shape of rete pegs. Discontinuity of basement membrane and loss of normal shape of basal cell layer. The underlying lamina propria, distinctive signs of inflammation by proliferation of inflammatory cells and island of epithelium in underling lamina propria.

Statistical analysis

A tabulation and statistical analysis of the gathered clinical data were conducted.

Clinical assessment

Mean of Gingival Index

Comparison of the mean of gingival index among different study groups

In the Comparable group, 25% of samples recorded score 0, and70% recorded score 1, and 5% of the sample recorded score 2. In the Stage I, 50% of samples recorded score 1, and 40% recorded score 2, and 10% of the sample recorded score 3. In the Stage II, 40% of samples recorded score 1 while 45% recorded score 2, and 15% recorded score 3.In the Stage III, 20% of samples recorded score 1 while 70% recorded score 2, and 10% recorded score 3 as seen in the following table:

Table 1: Gingival Index scores for each group are compared

Group	Score 0	Score I	Score II	Score III
Comparable	25 %	70%	5%	0%
Stage I	0%	50%	40%	10%
Stage II	0%	40%	45%	15%
Stage III	0%	20%	70%	10%

Comparison of the mean of Periodontal Probing Depth (PPD) among the different study groups:

The average depth of probing was (1.85±0.75) in the Comparable group. In the stage I, the mean of PPD was (3.80±0.62) and then higher values were showed in the stage II (4.55±0.60) and stage III (5.30±1.26).

The mean of PPD differed statistically significantly between the comparable group and all groups, the mean of PPD didn't exhibit any statistically significant variation among the subsequent stages: Stage I and Stage II and Stage III.

For intergroup comparison, small letters were used, and means with distinct superscript differ statistically significantly at $P \leq 0.05$. ** Overall p value of Inter comparison. According to the results of One-way ANOVA, the mean of the PPD value varied significantly between all groups, as indicated by the overall p-value of 0.00 (P >0.05) as seen in the following table:

Table 2: Mean ± Standard Deviation (SD) of PPD (mm) for all groups.

	Stage I	Stage II	Stage III	P-value**
1.85±0.75a	3.80±0.62b	4.55±0.60bc	5.30±1.26cd	0.00

Discussion

The test group's superior clinical improvements in terms of PPD decrease, CAL gain and inflammation reduction when compared to the closest control group were supported by the histology data, which further highlights the potential clinical importance of these findings.

In this study, stage I and the normal tissue group's basement membrane appeared normal; however, periodontitis in stage III ' basement membrane indicated a loss of continuity in several areas. These findings were in line with those of an earlier study, which found that when periodontal inflammation caused overgrown tissues to compare favorably to control tissues, there were discontinuities in the basement membrane.[11]

These results concur with those of Tanaskovic and Stankovic that they found the amount of gingival epithelium increased with direct proportion to the degree of the inflammation. Inflammed gingival tissue may be caused by an imbalance in tissue homeostasis and an increase in inflammatory cytokines like interlukin (IL)-1, IL-3, IL-6, and IL-8, which stimulate human keratinocytes proliferation in vitro.[12]

A decrease in keratinization was seen in this study, particularly in the subgroups with moderate and severe periodontitis. These results provide credence to the theory that a decrease in keratinization was directely correlated with the degree of the inflammation. The granular cell layer in the moderate and severe periodontitis subgroups collapsed and changed into a superfacial cell layer as a mechanical defence mechanism to make up for the thinner keratin layer.[13, 14]

The current study revealed that the moderate and severe periodontitis subgroup's basement membranes had lost some of their continuity, although the normal tissue group and the mild periodontitis subgroup's basement membranes appeared normal. These outcomes agree with what kantarci discovered. He noted that, in comparison to control tissue, enlarged tissue with periodontal inflammation had discontinuities in the basement membrane. Furthermore, as the integrity of the basement membrane was compromised, bacterial pathogens can more easily infiltrate the underlying tissues, these results shed light on the function that epithelial mesenchymal transition plays in the pathophysiology of periodontitis.[15, 16]

One of the most common inflammatory immunological diseases of the oral cavity is periodontitis. It results in the deterioration of periodontal tissue and is caused by a particular pathogenic bacteria-host interaction.[17]

Clinical periodontal parameters used in the present study assessing the periodontal conditions are Gingival Index (GI) and PPD. These periodontal indices have proved to be useful means of screening the periodontal condition, this

traditional method used for diagnosis, is still recommended today where tooth by tooth charting, measure PPD and gingival recession to accurately determine the stage of the periodontal disease. Identify several clinical criteria to locate loss of attachment and any tooth mobility.[18]

According to the current study's clinical findings, most patients had a gingival index score of 1, with the similar group recording the highest value at 70%. These may indicate that gingival tissues undergo an inflammatory process perior to clinical detection, which might be explained by a higher concentration of periodontal ligament bacteria in the nearby subgingival biofilm.

Statistical means of CAL and PPD were increased by increasing the stage number; this can be explained that the diagnosis and the severity levels of periodontitis were determined. Using the clinical characteristics of bleeding upon probing, CAL, and PPD.[19]

For CAL and PPD, According to Ramenzoni and Tomás, there was statistically significant difference between the comparable group and every stage and also reported higher mean CAL and PPD when comparing the people with periodontitis to those without it.[20]

For CAL and PPD, The overall P-value for intragroup comparison was highly significant. This is in accordance with Korte, clinical measurements can be used to assess the severity of the periodontitis and how well a treatment is working.[21]

Conclusions

The health of the gingival tissues and the degree of periodontitis were directly correlated. In case of the increasing of the gingival inflammatory processes led to the increasing of angiogenesis and inflammatory cell infiltration in the connective tissue and vice versa.

Declarations

Conflict of Interest

The authors have no financial conflicts of interest.

Funding

No fund.

Data availability

All data generated or analyzed during this study are included in this published article.

Ethical approval and consent to participate

The current study was executed according to the ethical guidelines set forth by the Research Ethics Committee of the Faculty of Dental Medicine for Girls, Al Azhar University, Egypt, under approval Final Code: REC-PD-24-18 on August 2024.

Competing interests

The authors declare no competing interests

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