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Evaluating the Effect of Scaling and Root Planing on the Salivary Levels of IL6 and RANKL

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Abstract

Periodontitis is a chronic inflammatory disease affecting the teeth' supporting structures. Scaling and root planing (SRP) is a foundational non-surgical periodontal therapy that aims to disrupt the subgingival biofilm and facilitate the healing of the periodontal tissues. The influence of SRP on salivary levels of inflammatory biomarkers, such as interleukin-6 (IL-6) and receptor activator of nuclear factor kappa-B ligand (RANKL), is not fully understood.

Purpose: The purpose of this study is to evaluate the effect of non-surgical periodontal treatment (scaling and root planing) on the salivary levels of IL-6 and RANKL

Methods: This clinical trial study, conducted with meticulous attention to detail, included 30 participants with periodontitis. Clinical parameters (plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL)) were examined at the baseline visit, after seven days, and after 30 days of non-surgical periodontal treatment. The salivary levels of interleukin-6 (IL-6) and receptor activator of nuclear factor kappa-B ligand (RANKL) were measured and compared before and after treatment, ensuring a comprehensive understanding of the subject.

Results: All clinical periodontal parameters were reduced (PI, BOP, PPD, and CAL) with a significant difference between baseline and the second visit scores (p= <0.0001). The salivary concentration of IL-6 and RANKL was decreased in all patients, with a significant difference between the baseline and the second visit measurement (p= <0.0001).

Conclusions: Scaling and root planing significantly affect the treatment of periodontitis by improving periodontal parameters and reducing salivary IL-6 and RANKL concentrations.

Introduction:

Periodontitis, a multifactorial disease, is the most prevalent oral inflammatory disease. It destroys the alveolar bone and causes subsequent tooth loss. The complicated etiology of periodontitis suggests that a vulnerable host and a

bacterial challenge are both participants in the disease's development (1,2). The penetration of bacteria into the gingiva triggers an immunological response from the host and initiates inflammation. This inflammation then leads to the destruction of the underlying periodontal tissues and the loss of alveolar bone (3,4). The primary objectives of periodontal treatment are to reduce inflammation, inhibit deep tissue invasion, and create an environment where gingival tissues can heal and be restored (5).

Treatment for periodontal disease involves managing the etiologic factors through various methods, including initial therapy, providing instructions and motivation for oral hygiene, and scaling and root planing. This is followed by a corrective phase of surgical treatment, with or without medical agents. Finally, a maintenance periodontal therapy phase. Each phase of treatment focuses on managing oral biofilm by treating factors that increase the risk and the underlying causes (6).

Scaling and root planing are dental procedures that effectively remove both supra- and subgingival deposits of calculus and plaque. Scaling involves eliminating plaque, calculus, and stains from the surface of a crown or root. On the other hand, root planing targets explicitly the removal of rough cementum or dentin that is impacted with toxins or bacteria or has calculus embedded into it. These contaminants can manifest as a coating of bacterial plaque and its associated toxic compounds, calculus, or defective cementum (7). In addition to calculus, the presence of endotoxin bound to cementum can affect the attachment and proliferation of gingival fibroblasts (8).

Scaling and root planing (SRP) are standard periodontal treatment methods; this study aimed to evaluate the effectiveness of non-surgical periodontal treatment (NSP) of periodontitis by investigating clinical and microbiological effects when recording the improvement in clinical parameters, significantly (PI, BOP, PD, and CAL) and evaluate the change in the salivary level of IL-6 and RANKL.

Interleukin-6 (IL-6) is essential in immune response, tissue regeneration, and metabolism. The rapid generation of IL-6 has a role in protecting the host during infection and tissue damage. Patients with periodontitis have been observed to have an increased concentration of salivary IL-6 (9,10). In addition, IL-6 was increased with stages of periodontitis with a significant difference, and the lowest mean value of IL-6 was in the healthy control group (11).

The receptor activator of nuclear factor kappa B ligand (RANKL) belongs to the TNF cytokine family and is essential for periodontal bone resorption. The expression of RANKL mRNA is significantly elevated in individuals with advanced periodontitis compared to those with mild periodontitis or healthy individuals. Furthermore, increased RANKL levels are associated with the abundance of P. gingivitis, a critical bacteria in periodontal disease, in periodontal tissue taken from clinical sources. Subsequent research has shown that bone resorption may be reduced by blocking the RANK/RANKL signal in rats with experimental periodontitis. These findings indicate that RANKL is crucial in periodontal resorption, and inhibiting RANKL can effectively prevent periodontal bone loss (12).

Saliva reflects overall health and can be used to monitor general wellness and the onset and progression of specific diseases like periodontitis (13). Biomarkers found in saliva from healthy individuals or those with systemic diseases can indicate health status, disease onset, and treatment responses. These biomarkers serve as early indicators of disease, making saliva a valuable tool in modern epidemiology (14).

This study's objective is to evaluate the effect of non-surgical periodontal treatment (scaling and root planing) on the salivary levels of IL6 and RANKL.

Materials and Methods

Study Design

This research was a clinical trial study conducted at the Teaching Clinics Department of Periodontics, University of Baghdad, College of Dentistry from January 2023 to August 2023. The study

protocol obtained ethical approval (Ref. No.:744 Date: 28/12/2022) from the Ethics Committee, College of Dentistry, University of Baghdad. All potential candidates were given informed consent forms to sign and were provided with a thorough description of the study's aims and objectives, and they were free to withdraw from the study at any time.

Sample size

Data from earlier studies were used to estimate the sample size (15). After employing hand instrumentation in a successful active periodontal therapy, periodontal pockets decreased from 7.64 ±1.76 to 5.68 ±2.3 mm. The necessary sample size was determined using the G*Power software (16). Based on the available data, about 20 participants were needed to detect a significant reduction in periodontal pocket depths with 80% power and a 5% significance level. This trial included 30 patients (n=30) to prevent dropout during follow-up.

The inclusion criteria

The inclusion criteria included a systemically healthy patient > 18 years old with unstable periodontitis (BOP> 10%, PPD ≥4 mm, and Interdental CAL ≥2 non-adjacent teeth, or Buccal or oral CAL ≥3 mm with pocketing >3 mm is detectable at ≥2 teeth (17).

The patient should have at least 20 teeth; it is necessary to have not taken antibiotics and anti-inflammatory medications for the last three months; the periodontal pocket depth should be 6-7 mm; and the patient should have at least two pockets.

The exclusion criteria

Patients with overhanging filling teeth or anomalies in their teeth, smokers, alcoholism, fixed prosthesis wearers, patients with chronic illnesses, immunocompromised patients, and women who were pregnant or nursing were among the exclusion criteria.

Clinical procedure

First visit (baseline): Each participant's full medical and dental history was taken and recorded on a special case sheet, and samples of unstimulated saliva were collected. Clinical periodontal parameters (full mouth PLI, BOP, PPD, and CAL) were measured. Dental impressions were taken to fabricate the stent, followed by motivation, instruction, and scaling. The same toothpaste and toothbrush were given to participants to avoid differences between the groups.

Second visit (after seven days from the first visit): Measurement of clinical periodontal parameters (PLI, BOP, PPD, and CAL) followed by root planing.

Third visit (after four weeks from root planing): Saliva samples are collected, followed by measurements of clinical periodontal parameters (PLI, BOP, PPD, and CAL).

Salivary Samples collection

Saliva samples were collected from patients at baseline visits before clinical examination and after four weeks of root planing. Subjects were asked to refrain from eating and drinking for 1-2 hours before the saliva collection. Unstimulated whole expectorated saliva (5 ml) was collected from each individual between 09:00 A.M. and 11:00 A.M. (18). Collected samples were placed on ice immediately and then frozen at -20 °C until analysis by ELISA.

Salivary Biomarkers Analysis

All saliva samples were thawed to room temperature before the experimental procedures and centrifuged at 1000×g for 20 minutes to eliminate cellular debris. Commercially available ELISA kits (SHANGHAI YEHUA Biological Technology Co., Ltd*)* were used to measure the concentration of salivary IL-6 and RANKL. The procedure was conducted following the manufacturer's instructions for each kit.

Results

Study population

Initially, over 100 patients diagnosed with periodontitis were examined at the clinic for eligibility according to inclusion/selection criteria. Nearly ten patients dropped out due to a lack of

commitment to appointment dates. Only thirty patients with periodontitis were recruited (12 women and 18 men; mean age: 37.53 ± 10.95, range: 20–62 years old).

Clinical periodontal parameters

Plaque index:

Plaque scores at baseline visit range (from 60.21 to 100.0, SD= 87.24 ± 12.49) and showed apparent reduction after one week range (6.000 to 23.80, SD=17.63 ± 4.07), while maximum reduction showed after 30 days (3.000 to 22.58, SD=11.24 ± 5.86). Also, the improvement in the treatment was compared to the baseline and second visit, which showed significant improvement (p= <0.0001) Table (1).

Bleeding on Probing:

At the initial examination, bleeding on probing scores ranged (28 to 90 SD= 58.36± 17.11). The bleeding scores were reduced after one week range (22.7 to 60.3, SD=36.01 ± 8.41), and more improvement was shown after 30 days (4 to 32.5, SD=11.23 ± 7.27) and showed significant differences between baseline and second visits (p= <0.0001) Table (1).

Probing Pocket Depth

Probing pocket depth at baseline visits for all patients ranged from (4 to 6, SD=5.43 ± 0.56) after one week showed nonclinical differences in the scores. Most of the periodontal pockets improved after 30 days (2 to 5, SD= 3.47 ± 0.63) with a significant difference (p=<0.0001) Table (1).

Clinical attachment loss

The range of CAL at the baseline visit (3 to 9, SD=5.57 ±1.48) for all patients. After one week of not registering any change in the score. While, after 30 days' slight improvement was shown (2 to 8, SD= 4.77± 1.41) with a significant difference (p=<0.0001) Table (1).

Determination of salivary biomarkers levels using ELISA

Interleukin-6 (IL-6) was evident high at baseline visit range from (119.4 to 320, SD=215.4 ±56.77) and improvement in the salivary IL-6 concentration showed after 30 days (14.72 to 58.87, SD=32.90 ±11.53) with significant difference (p=<0.0001) Table (1). In addition, RANKL was recorded at the baseline visit (21.13 to 88.01, SD=54.55 ±16.56) and also showed improvement in RANKL concentration after 30 days (1.510 to 13.82, SD=6.019 ±2.991) with significant difference (p=<0.0001) Table (1).

Discussion

It is well known that bacterial plaque plays an essential role in the initiation and progression of periodontal disease (19). Therefore, it is crucial to employ therapeutic methods that effectively target and manage biofilms to cure periodontitis. While this study primarily focused on subgingival instrumentation, it is essential to acknowledge that the patient's meticulous oral hygiene plays a crucial role in supragingival plaque control and the success of any periodontal therapy. The significance of bacterial plaque in initiating and advancing periodontal disease is widely recognized (20). This study investigated the effect of scaling and root planing in treating periodontitis. The main findings were an improvement in the clinical periodontal parameters (PI, BOP, PPD, and CAL) and a reduction in the concentration of salivary biomarkers (IL-6 and RANKL) after 30 days of the baseline visit. The clinical responses in this study agree with those reported by other investigators (21,22).

Plaque reduction is a prerequisite for controlling gingival inflammation (23). Despite recent technical developments, improved instruments, and new procedures, the effectiveness of both scaling and root planing and periodontal surgery remains dependent on plaque control, the quality of root debridement, and an effective maintenance program (24). In their study, David et al. (2016) found that scaling and root planing are effective treatments for many cases of periodontitis. They also concluded that adding conservative surgical approaches to scaling and root planing does not necessarily provide significant benefits in treating mild/moderate disease (25). Another study found that scaling and root

planing decreased probing pocket depths while increasing clinical attachment levels. Deeper sites exhibited the most significant PPD decrease and the most significant increases in CAL, whereas shallow sites had the most minor PPD reduction and the most CAL loss (26).

This investigation showed a notable decrease in the concentration of IL-6 between the baseline and the second recall visit. Their findings agree with previous studies, demonstrating notable enhancements corresponding to the reduction in IL-6 levels and the resulting decrease in inflammatory mediators. This creates an appropriate environment for tissue repair and the development of new attachments at the healing sites (27).

As well known, RANKL is pivotal in destructive bone diseases and is significantly elevated in diseased tissues by the alteration of gingival cytokine profiles. RANKL is responsible for osteoclast activation by connecting the receptor activator of nuclear factor-kappa B (RANK) and initiating bone resorption (28). A previous study found an increase in soluble RANKL concentration in individuals with periodontitis compared to healthy controls (29). In the present study, the concentration of RANKL was significantly decreased at the recall visit (after 30 days) compared to the baseline concentration in all patients, with a significant difference. This result agreed with previous studies that scaling and root planing could improve clinical parameters and decrease the salivary concentration of cytokines and osteoclastogenesis-related factors (RANKL and OPG) after one year at a follow-up visit (30).

Some limitations in the present study, such as the short follow-up period and the use of saliva as a source of biomarkers, may affect the measurement due to the dilution effect and lack of site-specific information, which is better evaluated by using GCF. Selecting patients with periodontitis only without defining the stage and grade may also affect the outcome of treatment.

Conclusion

In summary, this study confirms the efficacy of non-surgical periodontal treatment, specifically scaling and root planing, in improving clinical parameters (PI, BOP, PPD, and CAL) and reducing salivary levels of IL-6 and RANKL. These findings support the role of these interventions in managing periodontitis by mitigating inflammation and promoting tissue healing, highlighting their significance in periodontal therapy. Further research could explore extended follow-up periods and more precise biomarker assessments to enhance understanding of treatment outcomes and optimize patient care strategies.

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Conflicts of interest:
The authors claim to have no conflicting interests.

Table 1: descriptive and statistical analysis of periodontal parameters and Salivary IL-6 And RANKL between visits

	visits	Total number	Minimum	Maximum	Mean ± SD	2nd visit vs. baseline
AGE/years		30	20	62	37.53 ± 10.95	----------
PI%	Baseline	30	60.21	100.0	87.24 ± 12.49	<0.0001
	1st visit	30	6.000	23.80	17.63 ± 4.07	
	2nd visit	30	3.000	22.58	11.24 ± 5.86	
BOP%	Baseline	30	28	90	58.36 ± 17.11	<0.0001
	1st visit	30	22.7	60.3	36.01 ± 8.41	
	2nd visit	30	4	32.5	11.23 ± 7.27	

CAL (mm)	Baseline	30	3	9	5.57 ± 1.48	<0.0001
	1st visit	30	3	9	5.57 ± 1.47	
	2nd visit	30	2	8	4.77 ± 1.41	
PPD (mm)	Base line	30	4	6	5.43 ± 0.56	<0.0001
	1st visit	30	4	6	5.1 ± 0.66	
	2nd visit	30	2	5	3.47 ± 0.63	
IL6 (pg/ml)	Baseline	30	119.4	320	215.4 ± 56.77	<0.0001
	2nd visit	30	14.72	58.87	32.90 ± 11.53	
RANKL (pg/ml)	Baseline	30	21.13	88.01	54.55 ± 16.56	<0.0001
	2nd visit	30	1.510	13.82	6.019 ± 2.991	

Sd: standard deviation, PI%: plaque index percentage, BOP: bleeding on probing percentage, PPD: probing pocket depth, CAL: clinical attachment level; paired t-test was used to compare the means

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