Effect of Periodontal Therapy Using Minocycline Gel On Gingival Crevicular Fluid Osteoprotegerin In Chronic Periodontitis

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Abstract: Background and Objective: The aim of this study is to analyze the levels of Gingival Crevicular Fluid Osteoprotegerin (GCFOPG) in patients with chronic periodontitis prior to and following nonsurgical periodontal therapy, with or without the application of minocycline gel 2% for a period of 32 weeks**.** GCF samples were obtained from 24 diseased sites of 12 periodontal patients prior to as well as at 8, 16, and 32 weeks following non-surgical periodontal therapy. Gingival Crevicular Fluid Osteoprotegerin (GCFOPG) was determined by enzyme-linked immunosorbent assay (ELISA). Periodontal treatment was found to improve all clinical parameters. In both treatments osteoprotegerin concentrations were significantly elevated, but it was found to be significantly increased in minocycline treated group. The data suggested that periodontal therapy increases the level of Gingival Crevicular Fluid Osteoprotegerin (GCFOPG). However, a strong relationship was found between its amount in GCF and the degree of periodontal destruction and the amount of inflammation.
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1. Introduction

Chronic periodontitis is an inflammatory disease affecting the supporting tissues of teeth. The expression of the disease results from the interaction of host defense mechanisms, microbial agents, environmental, and genetic factors. Various compounds, such as cytokines, have been detected in gingival crevicular fluid (GCF) and may be especially beneficial for diagnosing current periodontal status and addressing the effects of periodontal treatment. [1,2]

Periodontal disease has two distinct, but interconnected, etiologic components; periodontopathic bacteria and host mediated response to the specific causative bacteria and their metabolic products. Periodontal pathogens have been shown to produce a large number of biologically active molecules that may act directly on host tissues. In addition, subsequent production of various inflammatory and immune mediators by the host may cause further tissue and bone destruction. [3]

Bone is a complex tissue composed of cells, collagenous matrix, and inorganic elements. It provides many essential functions, including mechanical support, protection of vital organs, a microenvironment for hematopoiesis, and a depot for calcium and other minerals. The growth, development, and maintenance of bone are a highly regulated process. [4]

It was reported that the osteoblasts or bone marrow stromal cells have been shown to regulate osteoclast differentiation, survival, and activation by providing microenvironment similar to bone. The discovery of new members of the Tumor Necrosis Factor Receptor-ligand family has clarified the molecular mechanism of osteoclasts differentiation regulated by osteoblasts/stromal cells. [5]

Osteoprotegerin (OPG) is a soluble decoy receptor of Tumor necrosis factor family. It inhibits differentiation, activation, function, and survival of osteoclasts by competing inhibition of receptor activator of nuclear factor kB ligand RANKL (present on osteoblasts surfaces) to the RANKL receptor called RANK present on osteoclasts precursors. [6]

The regulation of the balance between bone breakdown and reformation is modulated, to a large extent, by the secreted soluble receptor OPG. It has been postulated that, by binding to RANK-L, OPG makes RANK-L unavailable to RANK, blocking osteoclast differentiation and survival. Therefore, osteoclast differentiation, maturation, function and survival, in addition to bone homeostasis, is regulated in vivo by levels of expression of OPG. The ratio of RANKL/OPG expression determines the amount of the osteoclasts formation, where a shift to higher ratio of RANKL/OPG may be a major cause of bone loss in many metabolic disorders, including osteoporosis and periodontitis. [7]

The main therapeutic approaches for periodontal disease include mechanical scaling and root planing, thereby removing the bacterial deposits from the tooth surface and shifting the pathogenic microbiota to one compatible with periodontal health.

However, the pocket anatomy is a significant limiting factor, because mechanical access may not always be possible. [3]

Adjunctive administration of systemic antimicrobials has been useful in treating periodontal pockets. However, the doses necessary to achieve sufficient local concentrations of antimicrobials in the periodontal environment might be associated with undesirable side effects and have no site specificity. Local administration, therefore, may be considered as an alternative to overcome these problems. [3]

Tetracyclines are antiprotienases used in the treatment of periodontitis. Along with antimicrobial activity, tetracyclines agents have the ability to inhibit neutrophils, osteoclasts, and matrix metalloproteinases that appear to be involved in the destruction of the periodontium. Tetracyclines have an anti-inflammatory action and may be bone-sparing through inhibition of osteoclasts. [8]

Minocycline is a semi-synthetic tetracycline derivative that in addition to its antimicrobial effect, may inhibit interstitial collagenase activity and bone resorption. [9] Inhibition of osteoclast formation, inhibition of osteoclast activity, and induction of apoptosis are pharmacologically significant actions of minocycline in inhibiting bone resorption. [10]

Moreover, minocycline was found to effectively augment Prostaglandin E2 (PGE2) production, and Interlukin-1 (IL-1) level.[11] Furthermore, it has an inhibitory effect on Interlukin-6 (IL-6) expression in osteoblasts. [12] It was reported that the minocycline inhibits Tumor Necrosis Factor Receptor- α (TNF-α) and Gamma Interferon produced by stimulated T-cells. In addition, it inhibits the ruffled border of osteoclasts which is produced in response to Parathyroid Hormone (PTH). It also has an inhibitory effect on vitamin D3. Hence in the absence of these specific stimulators of bone resorption, OPG is produced and thus may help in inhibition of bone resorption. [13]

The present study is designed to examine the effect of periodontal therapy using minocycline gel on gingival crevicular fluid osteoprotegerin in chronic periodontitis

2. Materials and Methods

Patients with chronic periodontitis were recruited into this randomized, longitudinal, split-mouth, interventional study, from patients referred to the Department of Periodontology, College of Dentistry, Qassim University.

The selection criteria were (1) Patients aged 35–65 years for males and 35–45 years for females; (2) Good general health with no history of systemic disease; (3) No medication was taken; (4) No periodontal therapy received in the preceding 1 year; (5) More than 20 remaining teeth; (6) Moderate to advanced periodontal disease as evidenced by multiple sites with a probing depth of 5mm or more, extensive radiographic bone loss and bleeding on gentle probing; (7) Pregnant or lactating females were excluded. Postmenopausal females or others on estrogen therapy were excluded. [14] Informed consent was obtained from each patient prior to enrolment in this study, and ethical approval was obtained from the Qassim University ethics committee.

In each patient, two quadrants of either the mandible or maxilla were randomly assigned as experimental. Sites in one experimental quadrant received nonsurgical periodontal treatment consisting of oral hygiene instructions, scaling and root surface debridement followed application of 2% minocycline hydrochloride gel* in the periodontal pockets (group I), while the contralateral sites received nonsurgical periodontal treatment consisting of oral hygiene instructions, scaling and root surface debridement only (group II). The gel applications were repeated at 2 and 4 weeks after the first application

At 8, 16, and 32 weeks following treatment, the dentition received supragingival polishing with a rubber cup and pumice.

Prior to as well as 8, 16, and 32 weeks following periodontal therapy, a GCF sample was taken from each test site. The following clinical measurements were also evaluated: (1) Plaque index PI, according to Silness and Löe [15] (2) Gingival index (GI), according to Löe [16], (3) Probing pocket depth (PD), and (4) Clinical attachment loss (CAL), to the nearest millimeter with a Williams probe.

GCF Sampling

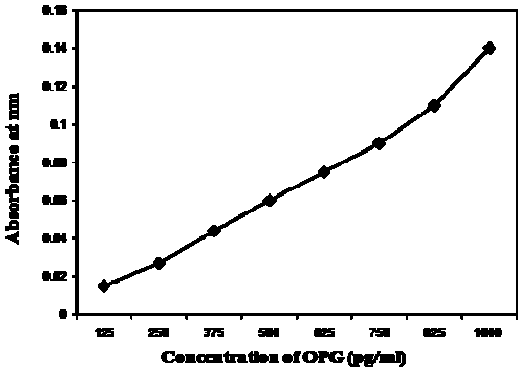
Both experimental quadrants were isolated with cotton rolls, and all clinically detectable supragingival plaque was removed using a curette without touching the marginal gingiva. Sites were gently dried with an air syringe, and a single sterile Whatmman Chromography filter paper 3MM cut into 2×13 mm strips were used for the GCF sampling. A paper strip was carefully inserted into the crevice until mild resistance was felt and left there for 30 seconds. Marks were placed gently on the limit of the wetted area of the strip just after removal from the gingival crevice. The strip was then placed in a sterile polypropylene tube containing 1mm saline, and was stored frozen at -20°C. Strips contaminated with blood or saliva was discarded.

Another paper strip was inserted into the same site 1 minute following the first sample. The

*Minocycline HEXAL® 50 mg filmtabletten. Hersteller, Salutas Pharma Gmbh, ein Unternehmen der HEXAL AG, Otto-von-Guericke-Allee 1, Barleban, Germany.

strip was then removed, dried for 15 minutes and immediately moistened by immersion into an alcoholic solution of 0.2% ninhydrine with the alpha amino acids in the fluid; the area of the strip which had taken up the crevicular fluid was stained pink. The length of the stained area of the paper strip was then measured to the nearest 0.5mm. The volume of GCF was calculated according to the calibrated curve. (Figure 1)

Figure (1) Standard curve of the concentration of OPG



Commercial Enzyme-Linked Immunosorbent Assay (ELISA Kit)† was used to analyze GCF Osteoprotegerin.

An anti-human OPG polyclonal coating antibody is adsorbed onto micro wells. Human OPG present in the sample or standard binds to antibodies adsorbed to the micro wells; a biotinconjugated polyclonal anti-human OPG antibody binds to human osteoprotegerin captured by the first antibody. Streptavidin-HRP (Hosre Radish Peroxidase) binds to the biotin conjugated anti-human OPG (sandwich technique). Following incubation, unbound biotin conjugated anti human OPG and Streptavidin-HRP is removed during a wash step and substrate solution reactive with HRP is added to the wells. A colored product is formed in, to proportion the amount of soluble human OPG present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human OPG standard dilutions and human OPG sample concentration determined. The average absorbance values are calculated for each set of duplicate standards and samples. Duplicates should be within 20 percent the mean. Samples have been diluted 1:2, thus the concentration read from the standard curve was multiplied by the dilution factor (X2).

† Bender MedSystems GmbH, Campus Vienna Biocenter 2, A-1030 Vienna, Austria, Europe

The final concentration can be calculated in each GCF sample by dividing the total amount of OPG in 1 ml saline by the volume of the sample.

3. Results

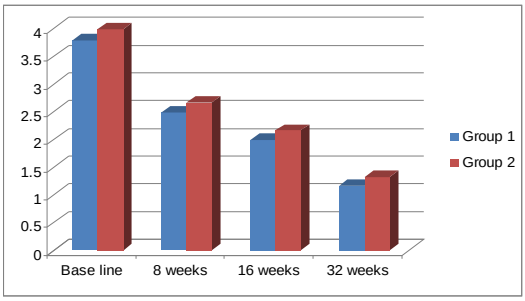
I- Clinical Parameters

Table (I) shows the comparison between the two studied groups regarding the probing depth (PPD) during the study period. It also reveals intragroup differences for this variable. In group I there was a statistically significant decrease in PPD at 8, 16, and 32 weeks when compared to the mean baseline value of 3.8± 0.41 mm, it was 2.5±0.55, 2±0.00 & 1.17±0.41 respectively. Also there was a statistical significant decrease in group II when compared to the baseline value of 4± 0.63 mm; it was 2.67±0.82, 2.17±0.48 & 1.33±0.52 at 8, 16, and 32 weeks (Graph 1).

Table (I): Intragroup evaluation and comparison of Probing Pocket Depth (PPD) in (mm) between the two studied groups throughout the study period.

PPD (mm)	Group 1 X ± SD	Group 2 X ± SD	Mann –Whitney test P- value
Base line	3.8 ± 0.41	4 ± 0.63	20.5 0.68
8weeks	2.5 ± 0.55	2.67±0.82	19.5 0.81
16weeks	2 ± 0.00	2.17 ± 0.48	21 0.63
32weeks	1.17 ±0.41	1.33± 0.52	21 0.63

Graph (1): The mean of probing pocket depth (PPD) in (mm) within the two groups throughout the study period.



The total % reduction after 32 weeks for group I was 69.21% and for group II was 66.75%.

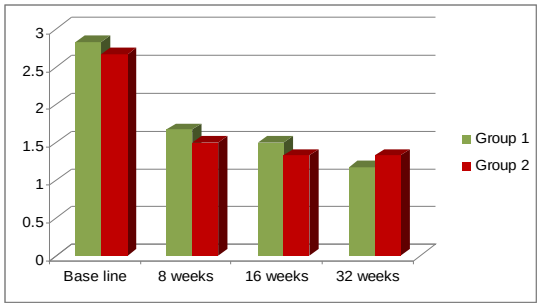
Table (II) shows the comparison between the two studied groups regarding the Clinical Attachment Level (CAL) during the study period. It also reveals intragroup differences for this variable. In group I there was a statistically significant decrease in CAL at 8, 16, and 32 weeks when compared to the mean baseline value of 2.83± 0.41 mm, it was 1.67±0.52, 1.5±0.55 & 1.17±0.41

respectively. Also there was a statistical significant decrease in group II when compared to the baseline value of 2± 0.67 mm; where it was 1.5±0.55, 1.33±0.52 & 1.33±0.52 at 8, 16, and 32 weeks (Graph 2).

Table (II): Intragroup evaluation and comparison of Clinical Attachment Level (CAL) in (mm) of the two studied groups throughout the study period.

CAL (mm)	Group 1 X ± SD	Group 2 X ± SD	Mann –Whitney test P- value
Base line	2.83± 0.41	2.67±0.52	21 0.63
8weeks	1.67±0.52	1.5± 0.55	21 0.63
16weeks	1.5 ± 0.55	1.33±0.52	21 0.63
32weeks	1.17±0.41	1.33±0.52	21 0.63

Graph (2): The mean of Clinical Attachment Level (CAL) in (mm) within the two groups throughout the study period.



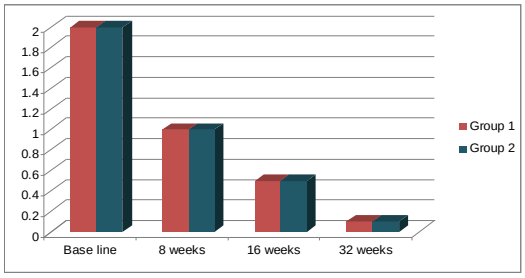
On comparing the % gain of the Clinical Attachment Level (CAL) in both groups at the end of the study period, it was found that the % gain in group I was significantly higher compared with group II; where it was 58.65% in group I and 33% in group II.

Table (III) shows the comparison between the two studied groups regarding the Gingival Index (GI) during the study period. It also reveals intragroup differences for this variable. In group I there was a statistically significant decrease in GI at 8, 16, and 32 weeks when compared to the mean baseline value of 2; it was 1, 0.5 & 0.1 respectively. Also there was a statistical significant decrease in group II when compared to the baseline value of 2; it was 1, 0.5 & 0.1 at 8, 16, and 32 weeks (Graph 3).

Table (III): Intragroup evaluation and comparison of Gingival Index (GI) scores of the two studied groups throughout the study period

GI SCORE	Group 1 Median score	Group 2 Median score	Mann –Whitney test P-value
Base line	2	2	18 1.0
8weeks	1	1	19 0.87
16weeks	0.5	0.5	22.5 0.47
32weeks	0.1	0.1	21 0.63

Graph (3): The mean of Gingival Index (PI) scores within the two groups throughout the study period.



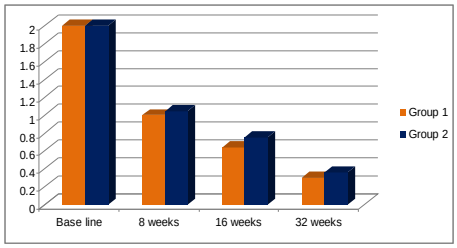
On comparing the % reduction of gingival index in group I and group II, no statistical significant difference between the two groups noted at 8, 16, and 32 weeks after treatment (% change of GI for both groups were 90% at 32 weeks). Also, no statistically significant difference between group I and group II were noted when comparing the percentage change throughout the study period.

Table (IV) shows the comparison between the two studied groups regarding the Plaque index (PI) during the study period. It also reveals intragroup differences for this variable. In group I, There was statistically significant decrease in PI at 8, 16, and 32 weeks when compared to the mean baseline value of 2; it was 1, 0.64 & 0.3 respectively. Also there was a statistical significant decrease in group II when compared to the baseline value of 2; it was 1.05, 0.75 & 0.36 at 8, 16, and 32 weeks (Graph 4).

Table (IV): Intragroup evaluation and comparison of Plaque Index (PI) scores of the two studied groups throughout the study period

PI score	Group 1 Median score	Group 2 Median score	Mann –Whitney test P-value
Base line	2	2	18 1.0
8weeks	1	1.05	27 0.15
16weeks	0.64	0.75	26.5 0.17
32weeks	0.3	0.36	24.5 0.29

Graph (4): The mean of plaque index (PI) scores within the two groups throughout the study period.



On comparing the % reduction of Plaque index in group I and group II, no statistical significant difference between the two groups noted at 8, 16, and 32 weeks after treatment (% change of GI for group I was 70 % at 32 weeks and 66% for group II. Also no statistically significant difference between group I and group II were noted when comparing the percentage change throughout the study period.

II- GCF osteoprotegerin (OPG) levels:

Table (V) shows comparison between the biochemical measurements of GCF osteoprotegerin (OPG) levels within and between the two groups throughout the study period.

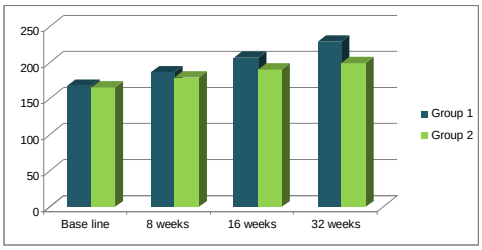
The mean baseline value in group I was 168.5pg/ml (±12.14). The mean value of OPG level was 186.50 pg/ml (±14.84) at 8 weeks, while at 16, 32 weeks it was 206.67 pg/ml (±12.66), and 229 pg/ml (±11.22) respectively. Group I showed statistical significant increase in OPG at 8, 16, and 32 weeks.

In group II the baseline mean level of OPG in GCF was 165.83 pg/ml (±13.47). Furthermore the value of OPG was 179.83 pg/ml (±8.59) at 8 weeks, while at 16 weeks it was 190.33 pg/ml (±7.79), and at 32 weeks the mean was 211.36 pg/ml (±20.95) (Graph 5).

Table (V): Intragroup evaluation and comparison of Gingival crevicular fluid levels of Osteoprotegerin (OPG) in (pg/ml) of the two studied groups throughout the study period.

OPG (pglml)	Group 1 X ± SD	Group 2 X ± SD	Mann –Whitney test P- value
Base line	168.5±12.14	165.83±13.47	20 0.8
8weeks	186.5±14.84	179.83±8.59	25 0.3
16weeks	206.67±12.66	190.33±7.79	31.5 0.02*
32weeks	229±11.22	199.8±6.68	36 0.002*

Graph (5): The mean of Gingival crevicular fluid levels of Osteoprotegerin (OPG) in (pg/ml) of the two studied groups throughout the study period.



On comparing the % increase of gingival crevicular fluid levels of OPG, the % increase of OPG levels in GCF in group I were significantly higher compared to group II throughout the study period. The % change was 11.01 for group I and 8.44% for group II at 8 weeks. Further increase in the % change of OPG level noted in group I 22.65% and in group II 14.77% at 16 weeks, the % change reached to 35.90 % and to 20.48 % for group I and group II respectively at 32 weeks.

4. Discussion

The ability to deliver an antibiotic in a biodegradable sustained release system directly into periodontal pockets offers a promising strategy for the treatment of sites that have not responded favorably to previous periodontal therapy. In addition to improving compliance over systemic antibiotics, biodegradable devices can overcome the systemic side effects, targeting the needed area, maintain constant therapeutic concentrations for prolonged periods of time, and are cost effective. Several locally delivered antibiotics have been used, but tetracycline class has proven to be the most effective when delivered locally, as an adjunct periodontal therapeutic agents. (17)

Minocycline HCL is a tetracycline derivative that is active against a broad spectrum of Gram-negative and Gram-positive anaerobes, including organisms implicated in chronic periodontitis. In addition to its antibacterial effect, the tetracycline analog minocycline has anti-inflammatory and proanabolic effects on tissue matrices; some of these effects may be mediated by stimulating alkaline phosphates activity, resulting in the expression of the mature osteoblast phenotype. (18) Moreover, minocyclines inhibits differentiation and activity of osteoclasts, and induce osteoclast apoptosis that may contribute to their ability to inhibit bone resorption, and regulate angiogenesis. (19)

In this study, non surgical periodontal treatment has improved all clinical parameters in both groups, yet this improvement was more significant in

the group where minocycline was used in conjunction with periodontal treatment. These results is in agreement with a study conducted by Lu H.K. et al. (2005) [20] who clarified the efficacy of the adjunctive use of subgingival minocycline application plus subgingival debridement (SRP) as compared with the results of one episode of SRP in the treatment of chronic periodontitis. Their results showed that by the end of the follow up period (18 weeks), the % change of PD for the minocycline was 74.36%, while the reduction rate of the control group was only 28.21% as compared to the baseline. Moreover, the clinical attachment loss showed statistical significant differences in results for week 18 (6.40±1.54 vs. 7.58±2.00mm) between the two groups, with the minocycline group experiencing more attachment gain than the control group. Furthermore, reduction in both GI and mean Interlukin-1β (IL-1β) content values in GCF at week 18 were also statistically significant in favor of minocycline group. Hence, they concluded that the local application of minocycline can be effective as an alternative adjunct to mechanical therapy in sites that respond poorly to a single episode of SRP. Similar results were also reported when minocycline microspheres were used as adjuncts to the scaling and root planning in the treatment of chronic periodontitis. [21-23]

Local delivered doxycycline hyclate has been shown to result in significant improvement in bleeding on probing, PD reduction and AL gain in cases with moderate (5-6mm) to deep (>7mm) periodontal pockets. [24, 25]

We suggest that the improvements in Group I treated with minocycline may be attributed to the fact that the Tetracyclines are considered as inhibitors of bone resorption. They inhibit osteoclast function and alter its adhesion apparatus (podosome) to bone surface. Moreover, in vitro tetracyclines have shown to prevent the formation of multinucleated osteoclast like cell.[26] In addition, tetracycline promotes the induction of osteoclast apoptosis that may inhibit bone resorption,[27] thus minocyclines are considered as a potent inhibitors of osteoclastogenesis. [28]

This superiority offered by minocycline may be attributed to that the subinhibitory concentration of minocycline that may suppress the adhesion and expression of bacterial virulence factors such as cysteine protease named gingipains, as well as fimbriae and Lipopolysaccharide (LPS), which may contribute to the pathogenesis of periodontitis. [29] It was reported that minocycline has the potential to modulate P.gingivalis activity and affect its physiology in suboptimal doses in subgingival sites. [30] Moreover, tetracyclines have the advantage of inhibiting proteases such as elastase which are responsible for matrix degradation.[31] Hence, minocycline application results in sufficient reduction in bacterial challenge to achieve significant better PDL health manifested by improvement in all clinical and biochemical parameters.

In addition, tetracyclines have proanabolic effects on connective tissues and bone repair. [32] Gomes P.S. and Fernandes M.H. (2007) [33] concluded that tetracyclines induce significant increase in the number of active osteoblasts cells that yielded a proportional amount of a normal mineralized matrix, suggesting a potential application in therapeutic approaches aiming to increase bone formation. Also, it was found that minocycline increases osteoid surface, mineralization surface, mineral apposition rate, and bone formation rate. Moreover, minocycline helps in expression of collagen I (main collagen in the tissue matrix) and/or the metabolism of osteoprogenitor cells. [34,35] It was stated that the anti-inflammatory properties of minocycline in protecting newly formed collagen may also be contributed to the effects of this drug. [30]

Sakai A. et al. (2006) [36] stated that the analysis of GCF may be especially beneficial for diagnosing current periodontal status and addressing the effects of treatment. Moreover, the identification of new markers in GCF may also contribute to elucidating novel mechanisms involved in periodontal diseases. Taubman et al. (2007) [37] reported that periodontal tissue destruction can be ameliorated by immunobiological interference with immune cell RANKL expression or function. They suggested that this new disease concept provides a foundation to build biological approaches to target RANKL production in periodontal lesions and thus give chance to increase OPG production.

Furthermore, OPG produces a marked inhibitory effect on the expression of cathepsin K which is the main enzyme involved in bone resorption. [38] It also acts as a survival factor for endothelial cells during periodontitis because it combats apoptotic cell death induced by microorganisms. [39,40] Thus. it is considered as a key molecule that positively regulates bone resorption. [41] Hence, a balance between OPG and RANKL may be associated with homeostasis of the periodontitis. [40,42]

Analysis of OPG factor in the present study was chosen to give an idea about the osteoclastogenesis process in periodontitis patients after the treatment application. It was found that at the baseline GCF OPG levels were reduced due to the inflammation and inhibitory effects of the bone resorbing factors, but it showed a significant increase throughout the study period in both treatment groups. OPG levels increased with %change 35.90 for group I, whereas in group II the levels of OPG increased with %change 20.48 at 32 weeks.

Bostanci N et al., (2007) [43] compared the levels of GCF of OPG, RANKL, and their relative ratio in GCF of healthy and PDL disease subjects. They found that the OPG concentrations in GCF were significantly decreased in all periodontal disease patients compared to health subjects. Moreover, the alteration of RANKL/OPG concentration ratio in GCF compared with health was significantly higher in all periodontitis patients. These results are in line with an earlier study by Mogi et al. (2004) [44] who speculated that the alteration of RANKL and OPG levels in GCF reflects the comprehensive biological responses that occur during the process of periodontitis. Furthermore, Bostanci N. et al. added that the OPG level in GCF was negatively correlated with all clinical parameters (PPD, CAL, PBI, and PI).

On the other hand, Lu H K et al., (2006) [45] identified the OPG/RANKL system in GCF and gingival tissues in patients with chronic periodontitis. They found that GCF levels of RANKL but not OPG were elevated in diseased sites of patients with periodontitis, moreover, the expression of OPG and RANKL showed no correlation with the disease severity. They attributed these results differences to the variant levels of sub-clinical inflammation among subjects or due to the differences in the sensitivity of various ELISA kits employed in each study.

The statistical significant increase of GCF OPG levels in both groups throughout the study period may be attributed to the pleiotropic effects of minocycline on osteotropic factors regulating the bone resorption such as PGE2, IL-1, IL-6, and TNF-α. [46-48] Moreover, minocycline has additional inhibitory effects on other bone resorbing factors such as [PTH, and 1, 25(OH) 2D3]. Minocycline help to increase OPG production and then return the balance between OPG and RANKL, thus inhibiting osteoclastogenesis and preventing more bone destruction, and osteoclast formation, giving a chance for bone formation. [49]

However, the statistical significant increase in the GCF OPG levels in minocycline group over group II may be due to the antibacterial action of minocycline. It was also reported that the LPS from several species of gm –ve bacteria have the ability to induce RANKL expression and decrease OPG production.(42) On the other hand, inhibition of RANKL function with OPG blocks the osteoclastogenesis, diminishing alveolar bone destruction, and reducing the number of periodontal osteoclasts after microbial challenge.[50] Hence, minocycline inhibits osteoclastogenesis produced by bacterial LPS that helps to decrease RANKL and increase of OPG, which may have a therapeutic value to prevent alveolar bone loss in periodontitis.

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