EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 227:239, JANUARY, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY
Submit Date : 24-08-2024 • Accept Date : 08-09-2024 • Available online: 10-01-2025 • DOI : 10.21608/edj.2024.315058.3172

UTILITY OF CREVICULAR PERIOSTIN AS A BIO-INDICATOR FOR PERIODONTAL HEALTH AND DISEASE

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ABSTRACT

Background and Objective: Periostin is a considerable structural protein and adhesion molecule that provides a pivotal function in maintaining tissue integrity. Therefore, the objective was to study its levels in the gingival crevicular fluid of individuals without any periodontal health issues in comparison with those who had periodontitis before and after periodontal therapy.

Subjects and methods: Crevicular sampling of periostin was conducted on 60 subjects that included 20 periodontally healthy individuals (Group I) and 40 patients, diagnosed with periodontitis, who were split into 2 groups. Group II received scaling and root planing (SRP), while Group III underwent SRP and intra-pocket placement of polycaprolactone nanofibers loaded by oxytetracycline hydrochloride (PCL/OTC). All participants underwent a clinical examination to evaluate their plaque and gingival indices, probing depth, and clinical attachment level. Additionally, their crevicular levels of periostin were analyzed biochemically at the beginning of the study and after 3 months.

Results: At baseline, the levels of periostin were found to be lower in patients with periodontitis compared to healthy individuals. After 3 months, there was a notable rise in the periostin levels, and an improvement in the clinical indices in the treated groups. Furthermore, groups II and III differed significantly in both periostin and clinical attachment levels in favor of group III treated by PCL/OTC.

Conclusion: The levels of crevicular periostin in periodontitis patients increased significantly after the periodontal adjunctive placement of PCL/OTC. Therefore, these molecular variations could reveal both disease susceptibility and activity, in addition to assessing the efficacy of periodontal therapy.

KEYWORDS: periostin, gingival crevicular fluid, periodontitis, polycaprolactone nanofibers, oxytetracycline hydrochloride.

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INTRODUCTION

Periodontal disease is a chronic inflammation resulting from polymicrobial dysbiosis of the associated biofilm, thus, affecting the composition and integrity of the periodontium and leading to deterioration of the tissues that support the teeth or what so-called periodontitis [1]. Moreover, the specific and complex interactions between the dysbiotic communities and host immune-inflammatory response deliver many inflammatory mediators and cytokines, which result in advanced periodontal collapse [2].

This periodontal destruction is fluctuant and characterized by bursts of disease activity alternated to periods of quiescence, however, current diagnostic tools and periodontal indices are unable to accurately evaluate the disease activity or the effectiveness of treatment [3]. Therefore, it is crucial to use omics sciences and find a valid indicator for efficient and reliable laboratory clinical evaluations that focus on the local inflammatory or regenerative responses [4].

Periostin is a protein consisting of 811 amino acids. It was first discovered in the osteoblasts of mice and is considered a matricellular protein [5]. It exhibits structural resemblance to fasciclin-1, an insect axonal guidance protein, and was earlier referred to as osteoblast-specific factor-2. Later, the name was modified to periostin because of its occurrence in the periosteum and periodontal ligament [6]. Periostin expression is stimulated by transforming growth factor-beta and bone morphogenetic protein-2 which regulate the interactions between the extracellular matrix and cells contributed to connective tissue repair [7].

Periostin has significant roles in various aspects of periodontal health, including the synthesis of collagen, adhesion, cell emigration, reactions to mechanical stresses, and remodeling by direct interaction with type I collagen and fibronectin [8]. Therefore, it renders an important protein for the periodontal tissue integral growth and maturation and is considered to play a vital function in organizing the periodontal ligament homeostasis [9]. It is also located amid the periodontal fibroblasts' cytoplasmic processes, close to collagen fibrils and cementoblasts [10]. Notably, periostin knock-out mice experimental studies have shown disturbed periodontal ligament remodeling and development of a phenotype resembling periodontal disease [11]. Furthermore, both *Porphyromonas gingivalis (P. g.)* lipopolysaccharide and tumor necrosis factor-alpha (TNF-α) are implicated in periodontitis and diminish the periostin production in human fibroblasts of periodontal ligament [9].

According to some studies, there is a notable correlation between the periodontal disease intensity and the periostin-reduced levels in the periodontal tissues [13], gingival crevicular fluid (GCF) [12, 14, 15, 16] and saliva [17]. Additionally, Aral *et al.* [18] found that the crevicular levels of periostin were lower in patients who have aggressive periodontitis compared to those with the chronic type and healthy subjects. On the other hand, compared to the periodontitis and peri-implantitis groups, the gingivitis and peri-implant mucositis samples had higher amounts of periostin [19]. In the meanwhile, Arslan *et al.* [20] noted that the level of GCF periostin was elevated in patients with periodontal disease and reduced after periodontal non-surgical treatment.

Regarding periodontal therapy, its goal is to modify or inhibit the periodontic microbial community to eradicate both periodontal pocket and subgingival infection, especially when the traditional therapeutic procedure is combined with the use of antimicrobial agents [21]. However, systemic administration of antibiotics does not completely eradicate bacterial species, as high doses are necessary to attain optimal medication concentrations in the GCF, leading to numerous harmful side effects [22].

Oxytetracycline hydrochloride (OTC) is a member of the tetracycline antibiotics family. It is a broad-spectrum antibiotic that works against various kinds of Gram-positive and Gram-negative bacterial species as it has a bacteriostatic action that inhibits the synthesis of bacterial proteins[23]. Additionally, tetracyclines are excreted in the GCF with a high capability for strong adsorption onto tooth surfaces, leading to improvement in the periodontal parameters [24].

The utilization of local delivery systems offers the notable advantage of ensuring the sustained administration of various medications at high dosages in the periodontally diseased sites. Therefore, they could maintain elevated concentrations of the drug in the crevicular fluid for a longer time and improve its curative efficiency [25]. Additionally, this delivery device could be easily inserted into the periodontal pocket and serve as a conduit for the efficient release and dispersion of any drug within the pocket [26].

Different nanoscale intra-pocket devices, for example, nanofibers, have evolved and may be an optimistic option for effective periodontal therapy. Nanofibers are identical biomaterials to the naturally occurring extracellular matrix, making them suitable for utilization as controlled delivery devices due to their exceptionally effective drug-loading capacity [27]. Furthering, polycaprolactone (PCL) polymer is a type of semi-crystalline aliphatic polyester with a recognized biocompatibility and biodegradability, that exhibits heat stability and favorable mechanical characteristics. Thus, it can be used in the development of local drug release systems for biomedical implementation [28].

Considering the existing controversy in the relationship between periodontal disease activity and periostin, and also the gap of information on its crevicular level [4], the objective was to determine and compare the CGF periostin levels in both healthy individuals and patients with periodontitis, both before and following periodontal therapy.

PATIENTS AND METHODS

Study population & design

A total of sixty participants were carefully selected from the Oral Medicine and Periodontology Clinic, Faculty of Dentistry, Sinai University. They included 34 males and 26 females, all aged between 33 and 55 years. Before obtaining informed consent, the participants were provided with a comprehensive explanation of the objectives and procedures. The Research Ethics Committee of the Faculty of Dentistry at Sinai University approved the protocol of this study (OMP-1-12-022).

All included participants underwent clinical and periodontal inspection after collecting their complete medical and dental histories. The study excluded individuals who had a medical history of systemic diseases, pregnancy, smoking or had reported the use of long-term anti-inflammatory drugs, antibiotics, or periodontal treatment within the preceding 6 months.

Based on the clinical and radiographic criteria outlined in the new periodontal classification scheme 2017 [29], those participants were divided into two main groups. The negative control group (Group I) included twenty subjects with clinically healthy periodontium, whereas the other forty participants were diagnosed as having stage II grade A periodontitis (clinical attachment level - CAL 3-4 mm and periodontal probing depth - PPD ≤ 5 mm with no tooth loss from periodontitis). These participants were divided equally into two groups, each consisting of 20 periodontitis patients. The patients were placed in either one of the therapy groups by a random assignment process using a coin toss process. Patients of the +ve control group (Group II) underwent conventional periodontal scaling and root planing (SRP) treatment only, while patients of the study group (Group III) underwent SRP in addition to the application of PCL nanofibers loaded with OTC (PCL/OTC).

For all participants, the baseline clinical parameters were documented and GCF samples were obtained after 24h to avoid contamination of those samples with the blood elicited during recording of periodontal indices. These periodontal indices included the gingival index (GI), plaque index (PI), periodontal probing depth (PPD), and clinical attachment level (CAL). PI was recorded for the mid-buccal, distobuccal, mesiobuccal, and palatal sites of each tooth [30]. GI assessment was conducted on the buccal, mesial, distal, and lingual gingival regions[31]. PPD and CAL were estimated in millimeters and evaluated across all teeth at six specifically designated locations [33]. After 3 months, the crevicular samples and periodontal indices were evaluated for patients diagnosed with periodontitis in groups II and III.

Treatment Protocol

Within 24 h of sampling collection, complete oral prophylaxis and full-mouth SRP were performed for all periodontitis participants in both groups II and III in the first week, using ultrasonic and hand instruments. All patients were instructed to avoid hard food intake, aggressive chewing and brushing or using any local and systemic antimicrobial agents throughout the study.

Moreover, to patients in group III, after meticulous isolation using cotton rollers and drying of periodontal pockets, PCL nanofibers loaded with OTC were gently administered until they filled the pockets without causing any bleeding. Those patients received instructions to brush twice gently at the selected sites and record any adverse effects or abnormal symptoms. Topical application of PCL/OTC was performed once weekly for three months. These PCL nanofibers were made using the electrospinning technique, and then loaded with 15 mg OTC according to the methodology of Dias *et al*. [34].

Collection of crevicular samples

GCF samples were collected from the mesiobuccal or distobuccal surface of the sites showing the most profound depth of periodontal pocket or gingival sulcus. At the baseline, two samples of GCF were obtained from the aforementioned sites for all groups, and after three months, samples were obtained from groups II and III. In the case that supragingival plaque was detected, it was scaled utilizing a sterile curette. The designated locations were subsequently isolated using sterile cotton rolls to avoid contamination by either oral microbiota or saliva. The crevicular fluid was collected by careful placement of the paper strips, until a slight resistance was detected, for 30 s employing the intracrevicular technique (Periopaper; ProFlow Inc., Amityville, NY, USA) [35]. Contaminated strips with blood or saliva were excluded.

Thereafter, the periopapers were transferred into sterile Eppendorf tubes filled with 100μl of phosphate buffer at a pH of 7.4. These tubes were promptly stored at a temperature of -20°C. Afterward, the sample was subjected to centrifugation at a speed of 3000 rpm for 15 minutes. The obtained sample was then utilized for the determination of periostin concentrations, which were assessed using a commercially accessible ELISA kit (Bioassay Technology Laboratory, Cat. No E3226Hu) following the instructions provided by the manufacturer. ELISA plate was pre-coated with the Human Periostin (POSTN) antibody. The POSTN, which was detected in the sample, was conjugated and bound to the antibodies that were coated onto the wells. Next, the biotinylated Human POSTN Antibody was conjugated and bound to the POSTN protein present in the sample. After that, Streptavidin-HRP was added and selectively attached to the Biotinylated POSTN antibody. Following the incubation period, the unbound Streptavidin-HRP was removed using a subsequent washing step. Following the addition of the substrate

solution, the developed coloration displayed a direct correlation with the quantity of Human POSTN present. The reaction was terminated by the incorporation of an acidic stop solution, and the absorbance was determined at 450 nm. The determination of crevicular periostin concentration was performed using a standard curve and presented as ng/30 seconds [36].

Statistical Analysis

The statistical analysis of the data under investigation was conducted using IBM SPSS (Statistical Package for Social Science), Software Version 20.0. In the analysis of parametric data, the one-way analysis of variance (ANOVA) test was employed to compare the means of three groups. Additionally, the paired sample t-test was utilized to compare the means of groups before and after treatments. In the analysis of non-parametric data, the Kruskal-Wallis test was employed to compare three groups, while the Mann-Whitney and Wilcoxon tests were utilized to compare groups both before and after treatments. The Chi-Square test was selected as the statistical method to examine the correlation between two or more qualitative variables. The Spearman correlation coefficient was also utilized. The level of statistical significance was established at $p < 0.05$.

RESULTS

Sixty participants were involved and divided into 3 groups; Group I (-ve control group) comprised 20 healthy individuals; whereas groups II (+ve control group treated by SRP) and III (study group treated by SRP and PCL/OTC) included 20 periodontitis patients for each. Measurements of periodontal parameters were documented, and crevicular samples were obtained during the initial assessment and three months following the treatment. The age of the participants in the study spanned from 33 to 55 with an average age of 35.9±7.3, 41.5±8.3, and 41.6±6.8 in groups I, II, and III; respectively. Regarding the gender distribution of those participants, group I included 14 males and 6 females, whereas group II involved 9 females and 11 males, and group III consisted of 11 females and 9 males. However, no statistically significant differences were observed between the different groups regarding either age (p=0.087) or gender (p=0.275) **(data unshown)**.

Considering the crevicular periostin, its baseline was found to be significantly greater in healthy participants than in periodontitis patients as shown in **Table 1** (p<0.001). After treatment, the levels of it showed a substantial increase in periodontitis patients (p*<0.001), nevertheless, those levels did not reach the normal in both treated groups (II and III). However, this increase was in favor of the periodontitis patients treated by SRP and PCL/OTC (group III) rather than those treated by SRP only (group II). Furthering, intergroup comparison analysis of GCF periostin levels at the baseline revealed a statistically significant variation among the different groups ($p1$<0.001), as well as group I and each of groups II and III ($p2$ & $p3$<0.001), however, there was no statistically significant distinction observed between groups II and III ($p4$=0.234). After treatment, there were statistically significant variations in those levels observed among the involved groups ($p1, p2, p3$ & $p4$≤0.001) as shown in **Table 2**.

Clinically, no adverse effects or unusual symptoms were recorded throughout the study period. Additionally, the mean values of the clinical parameters are displayed in **Table 3**. Regarding the periodontal indices, intragroup comparison before and after treatment showed a statistically significant difference in all measured values in both treated groups (II & III) at p*<0.001. Considering intergroup comparison analysis, there were statistically significant differences among all the studied groups in all parameters before and after treatment ($p1$<0.001). Moreover, intergroup

comparison analysis among each of groups (I & II) and groups (I & III) displayed statistically significant differences before and after treatment regarding each of PI, GI, PPD, and CAL (*p2 & p3*<0.001). However, there was no statistically significant distinction observed among groups (II & III), except for CAL after treatment (*p4=0.046*). Additionally, the decreased CAL was in favor of the periodontitis patients treated by SRP and PCL/OTC (group III) rather than those treated by SRP only (group II).

After treatment interventions, a significant inverse relationship was observed between periostin levels and each of PI and GI in group II and each of PPD and CAL in group III. However, these correlations were not statistically significant as shown in **Table 4**.

TABLE (1) Periostin (ng/30 seconds) levels before intervention in healthy and diseased groups.

	Median (Min-Max)	**P value**
Controls (n=20)	4.1 (3.295-5.589)	< 0.001
Patients (n=40)	1.47 (0.44-2.7)	

P*; p-value*, Mann-Whitney test *is used.*

TABLE (2) Periostin (ng/30 seconds) levels before and after intervention in healthy and diseased groups.

Groups	**Before**		**After**		**P* value** (Paired test)
	Median (Min-Max)	**P value**	**Median (Min-Max)**	**P value**	
Group I (-ve Control)	4.1 (3.295-5.589)	P1<0.001 P2<0.001 P3<0.001 P4=0.234	4.1 (3.295-5.589)	P1<0.001 P2<0.001 P3=0.001 P4<0.001	-
Group II (+ve Control)	1.47 (0.703-2.65)		2.71 (0.714-2.87)		<0.001
Group III (Study)	1.32 (0.44-2.7)		3.37 (2.2-4.39)		<0.001

P1*; P value between Group I, II and III*, P2*; P value between Group I and Group II*, P3*; P value between Group I and Group III*, P4*; P value between Group II and Group III*, P; P value of intragroup comparison*, Kruskal-Wallis Test *is used*, Wilcoxon Test *is used for paired test.***

TABLE (3) A comprehensive clinical evaluation of patient groups before and after the treatment.

		Before	P value	After	P value	P* value (Paired test)
PI	**Group I**	0.17±0.29	P1< 0.001	0.17±0.29	P1<0.001	
(Mean ±SD)	(-ve Control)		P2<0.001		P2<0.001	
	Group II	2.45 ± 0.278	P3<0.001	0.975 ± 0.288	P3<0.001	< 0.001
	(+ve Control)		P4=0.273		P4=0.108	
	Group III (Study)	2.35 ±0.225		0.843 ± 0.177		< 0.001
GI	**Group I**	0.19±0.3	P1< 0.001	0.19±0.3	P1= 0.001	
(Mean ±SD)	(-ve Control)		P2<0.001		P2<0.001	
	Group II	1.86 ± 0.237	P3<0.001	0.491 ± 0.253	P3=0.006	< 0.001
	(+ve Control)		P4=0.584		P4=0.379	
	Group III (Study)	1.82 ±0.186		0.42 ± 0.168		< 0.001
PPD	**Group I**	1.45±0.15	P1< 0.001	1.45±0.15	P1< 0.001	
(Mean ±SD)	(-ve Control)		P2<0.001		P2<0.001	
	Group II	3.41 ± 0.354	P3<0.001	2.51 ± 0.43	P3<0.001	< 0.001
	(+ve Control)		P4=0.181		P4=0.598	
	Group III (Study)	3.28 ± 0.34		2.69 ± 0.326		< 0.001
CAL	**Group I**	0	P1< 0.001	0	P1< 0.001	
(Mean ±SD)	(-ve Control)		P2<0.001		P2<0.001	
	Group II	2.75 ± 0.297	P3<0.001	2.14 ± 0.293	P3<0.001	< 0.001
	(+ve Control)		P4=0.441		P4=0.046	
	Group III (Study)	2.69 ± 0.327		1.94 ± 0.436		< 0.001

GI*, gingival index*; **PPD***, periodontal probing depth*; **CAL***, clinical attachment level*; **SD***, standard deviation*, **P1***; P value between Group I, II and III*, **P2***; P value between Group I and Group II*, **P3***; P value between Group I and Group III*, **P4***; P value between Group II and Group III*, **P****; P value of intragroup comparison*, **one-way ANOVA** *and* **Paired Sample T-test** *are used*

TABLE (4) The correlation analysis between periostin concentrations and clinical parameters in patient groups before and following the treatment.

		Group II (+ve control)		Group III (Study)	
		Before	**After**	**Before**	**After**
PI	***R***	0.008	-0.051	-0.186	0.020
	P	0.975	0.829	0.431	0.934
GI	***R***	-0.157	-0.094	-0.20	0.285
	P	0.508	0.693	0.398	0.223
PPD	***R***	-0.041	0.038	-0.129	-0.026
	P	0.863	0.874	0.587	0.914
CAL	***R***	-0.096	0.014	-0.246	-0.027
	P	0.687	0.954	0.295	0.909

GI*, gingival index*; **PPD***, periodontal probing depth*; **CAL***, clinical attachment level*; **Spearman correlation** *is used*

DISCUSSION

Given the importance of early diagnosis, the use of various proteins and enzymes; as biomarkers for chairside screening; has been assumed. Those molecules have a significant impact on either the early staging or advancement of periodontitis [9]. Periostin is a modulator protein that has a pivotal function in the cross-linking and arrangement of collagen and non-collagen extracellular matrix proteins [37]. It also has enhanced specificity compared to other proteins expressed in the periodontal ligament, making it a potential biomarker for several diseases [16].

Furthering, electrospun PCL-based nanofibrous formulations have attained significant success in several biomedical branches, such as nano-based drug delivery systems[38]. These systems offer a large specific surface-to-volume ratio and augment the drug loading capacity. They also increase the solubility and permeability of drugs and their half-life[39]. So, they are promising adjuvant modalities in periodontal therapy, especially when incorporated with antimicrobial agents to provide more effective release performance and treatment [34].

Accordingly, in the current study, crevicular periostin levels were evaluated in the participants with clinically healthy periodontium and in peri-odontitis patients treated either by SRP or SRP and PCL nanofibers loaded by OTC. Those participants were selected with an age range of 33-55 years to reduce the impact of age on periostin levels as there were no significant variations in either age or gender among the different groups. Moreover, the potential efficacy of periodontal treatment was assessed by analyzing the clinical parameters and measuring the crevicular periostin levels to realize the dynamics at the molecular level and tissue homeostasis.

The GCF was sampled using intra-crevicular filter paper strips as a non-invasive and easily applied method at the periodontal sites. Therefore, its analysis was considered a confident choice in determining the early changes indicative of disease onset and activity [35]. In the present study, crevicular periostin levels were notably decreased in periodontitis patients compared to periodontal healthy individuals at the baseline. These findings agreed with the results of Sophia *et al.* [16], who reported that the crevicular levels were significantly lower in individuals with chronic periodontitis compared to those with gingivitis and healthy periodontium. Moreover, those levels were markedly reduced in the patients affected with aggressive and chronic periodontitis in comparison with the non-affected participants [18,40]. Therefore, it was thought that the delayed healing of the tissue caused by periodontal inflammation could lead to a decrease in periostin levels and accelerate the advancement of the disease [13].

Indeed, many studies [12, 13, 16, 18, 40. 41] reported that the level of crevicular periostin was gradually decreased by increasing the severity of periodontal disease and those low levels were explained by two mechanisms. The first mechanism is attributed to the ability of the defense against periodontal pathogens can modulate the expression of this biomarker. The second mechanism relates the previously mentioned decline to a reduction in periodontal ligament cells, which are amongst the main contributors of periostin production, in the advancement of the disease [14].

Regarding periodontal therapy, periostin levels were increased after treatment in comparison with the baseline levels in both groups II and III of periodontitis patients. This was consistent with other studies [42,43,44] that reported that GCF levels of periostin were high post-conventional periodontal therapy. This was explained by its function in controlling fibronectin during the healing process of wounds [45]. Another study used low-level laser treatment as an adjunctive to non-surgical periodontal therapy. The levels of periostin in the gingival fluid were greater in the group with healthy periodontium compared to the group with chronic periodontitis before treatment. After treatment, these levels dramatically increased compared to the initial values [15]. Additionally, the levels of GCF periostin were found to be higher in patients with

chronic and severe periodontitis who underwent open flab debridement [14]. Certainly, the resolution of inflammation after periodontal therapy increased the expression of this protein which promotes the immigration and growth of cells and also stabilizes the extracellular matrix to aid and foster the healing process [14].

Further, Padial-Molina *et al*. declared a reduction in the messenger RNA transcription and the amount of periostin in periodontal ligament fibroblasts after being subjected to *P.g*. and TNF-α *in vitro*. This bacterial challenge might increase the upregulation of periostin and its levels in the early exposure, which is subsequently followed by a notable decrease as the disease advances [9]. Therefore, the rise in periostin levels in Groups II and III compared to the first measurement was likely because of periodontal therapy that decreased both *P.g* and TNF-α implicated in the pathogenesis of periodontitis.

Contrary to the presented results, Arslan *et al*. [20] reported the lowest periostin levels in healthy gingiva, followed by gingivitis, whereas the highest levels were in periodontitis at the starting point. Furthermore, there was no notable distinction observed between gingivitis and periodontitis, and the crevicular levels were decreased after SRP at the end of the third month. This discrepancy might be attributed to the different stages and grades of periodontitis as the patients had stage II-III grade B periodontitis. Additionally, the crevicular samples were obtained from single-rooted teeth.

Moreover, Sari *et al*. [46] found higher crevicular levels of IL-1β, periostin, and IL-39 levels in periodontitis and gingivitis than in healthy periodontium. The inconsistency could be owing to the variations in the progression of the disease in the areas of crevicular sampling, which were obtained from patients suffering from stage III-IV (severe) periodontitis. Additionally, the levels of periostin were shown to be higher in samples from individuals with peri-implant mucositis and gingivitis compared to those with peri-implantitis and periodontitis; however, those values were not compared by healthy controls [19].

This conflict may be also attributed to the binary actions of periostin as an extracellular matrix and a matricellular protein. The protein accumulates in areas of inflammation where fibrosis is present. Additionally, it stimulates both immune and nonimmune cells as a matricellular protein, so intensifying the inflammatory response [20]. Periostin also increased in the diseased sites because of the perpetuation of bacterial infection and the resulting immunological response as it was raised as a defensive reaction to inflammation to enhance tissue repair [46].

Regarding the recorded periodontal parameters, all the mean values were comparatively lesser in healthy subjects of group I than in periodontitis patients of groups II and III and the outcome differences were statistically considerable at time zero. This finding was comparable to previous studies in which the mean PI and GI values [47] in addition to PPD and CAL values [48] were high in patients suffering from chronic periodontitis in comparison with healthy subjects. Following periodontal therapy, a comparison between groups II and III revealed a statistically significant difference in all recorded parameters. Moreover, all these parameters were decreased in the periodontitis groups and this improvement showed statistical significance when comparing group I to both groups II and III.

Of course, the improvement in these periodontal parameters is because of the beneficial impact of periodontal therapy and the removal of subgingival plaque microbiota [49]. Therefore, there were not any significant differences of statistical importance between groups II and III in all clinical values except in CAL where more gain was achieved in group III. This is attributed to the beneficial effect of adjuvant PCL/OTC nanofibers and the limited effect of SRP alone especially in inaccessible areas with complex

anatomy. Moreover, it is worth mentioning that the increased periostin levels were also in favor of group III.

This result emphasized the valuable usage of adjuvant therapies to enhance the efficacy of SRP and resolve inflammation. In addition to the documented antibacterial efficacy of OTC, it can also inhibit collagen degradation and bone resorption, thus allowing favorable tissue remodeling [24]. Consistent with this finding, other studies also reported a significant decrease in periodontal parameters in patients treated with PCL nanofibers loaded with oxytetracycline hydrochloride [38] and doxycycline [50] in comparison to SRP alone in the treatment of chronic periodontitis.

These results were supported by Dias *et al.* study [34] which stated that PCL nanofibers loaded with OTC were effectively produced by the electrospinning methods and exhibited continuous drug release for 10 hours, resulting in the complete release of 100% of OTC. This local delivery system also displayed excellent antibacterial activity against a diverse bacterial culture consisting of Gram-negative anaerobic bacteria implicated with periodontal disease. To some extent, these outcomes were also owing to the biocompatibility and biodegradability of PCL and its good mechanical and thermal stability. Hence, PCL/OTC nanofibers possess significant potential as a medication delivery system for the treatment of periodontal diseases.

Also, an adverse correlation was observed between periostin levels on one hand and plaque and gingival indices in group II and each of PPD and CAL in group III on the other hand. This aligns with the findings of other studies [12, 15, 18] that similarly observed an inverse relationship between GCF periostin and clinical parameters in patients with chronic periodontitis. This could indicate that periostin may play a protective role in the periodontal hemostasis following periodontal therapy.

CONCLUSION

Periostin expression can clarify diverse pathways in either the pathogenesis or healing of periodontal diseases. Thus, it affords a promising, reliable, and authentic inflammatory biomarker for diagnosing and evaluating periodontal therapy and its following outcomes. Furthermore, the use of PCL/OTC nanofibers as an additional treatment enhances the clinical parameters and elevates periostin levels in patients with periodontitis. Additional research involving a larger number of participants and different periodontal diseases staging and grading as well as assessing the long-term effects of various treatments, is necessary to gain a more comprehensive understanding of periostin expression patterns during the healing process of periodontal disease and its full role in the development of periodontal diseases.

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