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IMPACT OF SMOKING ON GINGIVAL CREVICULAR FLUID AND SALIVARY PERIOSTIN LEVELS IN PERIODONTITIS PATIENTS FOLLOWING NON-SURGICAL PERIODONTAL THERAPY

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ABSTRACT

Background: Smoking has been identified as a major risk factor for periodontal diseases which results in its rapid progression. Periostin is a matricellular protein that is highly expressed in periodontal ligament and was found to be downregulated during periodontal disease. The aim of this study was to evaluate the effect of non-surgical periodontal therapy on clinical parameters as well as GCF and salivary periostin level in smoker and non-smoker patients with stage II & III periodontitis.

Methods: Sixty subjects participated and divided into 20 periodontally healthy participants three study groups: group I included 20 periodontally healthy participants, group II included 20 smoker patients with stage II-III periodontitis and group II included 20 non-smoker patients with stage II-III periodontitis. Both groups II and III received phase I periodontal therapy (subgingival scaling and root planing) . Clinical parameters (PI, GI, PD, CAL) were recorded and GCF and salivary samples were collected from both periodontitis groups at baseline and again 3 months after phase I therapy. Samples were then analyzed using ELISA for periostin levels.

Results: Non-surgical periodontal therapy resulted in significant improvement in clinical parameters and increase in GCF periostin level while reduction in salivary periostin level in both smokers and nonsmokers with more significant improvement in the non-smokers group.

Conclusion: In smoker periodontitis patients, periostin could act as a potential biomarker not only for disease progression activity, but also could be targeted for faster tissue repair and more attachment gain.

KEYWORDS: Periostin, smoking, non-surgical therapy, GCF, Saliva, Periodontitis

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INTRODUCTION

Periodontal diseases are a group of conditions affecting the supporting structures of the dentition ***(Pejcic et al, 2007)***. Periodontitis results from interaction between biofilm bacteria in the dental plaque and the host immune-inflammatory response. This response is part of the host defense mechanism, however substantial damage occurs in periodontal tissues with resultant periodontal ligament fibers destruction and loss of clinical attachment alongside alveolar bone loss .The host response is coordinated by many mediators such as prostanoids, cytokines and matrix metallo-proteinases MMPs ***(Preshaw & Taylor, 2012).***

Periostin (POSTN); takes its name from being expressed in periodontal ligament and periosteum ***(Kruzynska-Frejtag et al, 2004)***; is a matri-cellular protein that impacts cell matrix interactions, cell functions, wound repair, tissue remodeling, collagen type I maturation in periodontal ligament and regulation of bone formation ***(Kudo, 2011)***. Biologically, POSTN functions comprise conserving connective tissue in both statuses of health and disease ***(Yamada et al, 2014).*** It has a role in triggering cell activity through binding to its surface receptors, therefore applying its effects of biological nature ***(Romanos et al, 2014)***. POSTN that is released by fibroblasts, exists in numerous tissues, saliva, serum, and gingival crevicular fluid GCF ***(Romanos et al, 2014).***

In chronic periodontal disease, PDL cells proliferation and differentiation are significantly declined, the tissue integrity is impaired and both tissue regeneration and healing are seriously compromised. Under such circumstances, periostin serves as a marker for reconstructive cellular matrix interactions and cell behavior in matrix biomechanics. It preserves hemostasis and protects connective tissue integrity ***(Padial-Molina et al, 2014)***.

Progression and severity of periodontal disease depends on the complex interactions between several risk factors such as microbial, immunological, environmental and genetic factors, as well as the utilization of tobacco products has long been linked to periodontal disease.

Cigarette smoking alone does not initiate periodontal disease however, it influences its rate of disease progression. In smokers, once periodontal disease starts, smokers lose bone at a faster rate than do non-smokers. This is because of the influence of nicotine on vasoconstriction of the blood vessels, minimizing blood supply reaching the tissues and reduceing the intake of oxygen by hemoglobin, thus in turn retards the body's ability to fight the infection and bone resorption ***(Soben, 2009).***

The effect of smoking status on periostin expression in both GCF and saliva has never been investigated before. It is unknown whether salivary and GCF periostin concentrations are altered in smokers' periodontitis patients compared to their levels in non-smokers (either periodontitis patients or healthy controls) in response to non-surgical periodontal therapy.

In the present study, the GCF and salivary POSTN levels in patients diagnosed with periodontitis either smokers or non-smpkers will be assessed and compared with each other and with those of healthy controls, thus POSTN can play an important role in the pathogenesis of periodontal diseases. This can provide a novel insight into utilizing periostin levels in saliva and GCF in smokers as a diagnostic periodontal disease marker, and also biomarker guided periodontal therapy in the future.

SUBJECTS AND METHODS

Study design

This study is a three-arm non-randomized clinical trial with allocation ratio 1:1:1

Study population

This clinical trial was conducted in Periodontology clinic, Faculty of Dentistry, Cairo University, Egypt

on a total of 60 participants. Participants were divided into three groups; each group consisted of 20 participants: Group I consisted of 20 subjects with healthy periodontium; Group II consisted of 20 nonsmoker patients with stage II-III periodontitis; Group III consisted of 20 smoker patients with stage II-III periodontitis.

Participants were recruited by consecutive sampling from the outpatient clinic, Department of Periodontology and Oral Medicine, Faculty of Dentistry, Cairo University between September 2020 and March 2021. Screening of participants was continued until the required sample was achieved.

This clinical trial was registered in U.S. National Institutes of Health Clinical Trials Registry, Clinicaltrials.gov ID: NCT03728244.

Sample-size calculation:

On the basis of the study by ***Arslan et al., 2020*** , calculation of the sample size was done using G-power software, based on the power analysis with α error of 5% and 80% power, and the sample size expected for this study was 54 (18 in each group) . This number was increased to 60 (20 in each group) to compensate for potential drop out along the study period.

Research ethics approval:

The study protocol was approved by the Ethics Committee of Scientific Research, Faculty of Dentistry in Cairo University in 25 June 2021, with approval number 18-12-17. The aim of the study, detailed procedures and follow up visits were explained in details to all participants, and then all subjects agreed to participate in this trial, signed a written informed consent.

Eligibility criteria:

Inclusion criteria included : Group I : Periodontally healthy group included subjects with full mouth Bleeding on probing score < 10 %, pocket depth (PD) ≤ 3 mm , no CAL, no radiographic bone loss ***(Trombelli et al., 2018)*****;** Group II : non-smoker patients having Stage II-III Grade B periodontitis who had minimally two non-adjacent teeth with sites measuring 3 to 4 mm or ≥ 5 mm interdental CAL and 15% to 33% or radiographic bone loss that extends to middle third of root and beyond ***(Tonetti et al., 2018)***; Group III: smoker patients with Stage II-III Grade B periodontitis with the previously mentioned criteria.

Exclusion criteria included : Subjects with known systemic disease; patients that received any drug which may affect periodontal wound healing within the past 6 months like antibiotics or anti-inflammatory drugs, patients under drug therapy that could affect periodontal status like long-term anti-inflammatory drugs or contraceptives; pregnant or lactating women; patients who refused to provide written informed consent.

Clinical periodontal examination and treatment protocol:

Full mouth periodontal charts and periapical x-rays were obtained for each patient to confirm the diagnosis and to ensure that every patient was suitable for the selected group according to the inclusion and exclusion criteria set before for each group.

Group I with healthy periodontium did not receive any periodontal therapy; while groups II and III received full mouth supra-gingival scaling performed using ultrasonic device* with supra-gingival inserts for gross scaling followed by sub-gingival debridement using Gracey's curettes**. Preparation of each patient was completed in a single visit. Then each patient was given strict instructions regarding proper oral hygiene including: teeth brushing twice daily by soft toothbrush using circular scrub technique and interdental cleansing

* NSK non-optic ultrasonic scaler, Kanuma-shi, Japan
** Nordent curettes; Nordent Manufacturing Inc, USA.

according to the size of interdental embrasure and rinsing with 0.125% Chlorhexidine HCL mouthwash (Hexitol®*) to be used twice daily for 2 weeks.

For periodontitis patients (group II & III), the following clinical parameters were registered at baseline only for group I and at baseline and again 3 months after treatment for groups II & III : plaque index (PI) ***(Silness & Löe, 1964)***, gingival index (GI) ***(Löe & Silness, 1963)***, PD and clinical attachment level (CAL). PD and CAL were measured on six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) of the teeth with a William's graduated periodontal probe. All clinical measurements were accomplished by a single investigator (A.A)

Collection of GCF and salivary samples:

GCF & salivary samples were collected at baseline only for group I and at baseline and 3 months after non-surgical periodontal therapy in groups II & III.

GCF sample were obtained from the mesio-buccal or disto-buccal surfaces of teeth with the deepest PDs as follows: the selected sites were isolated by cotton rolls, gently water rinsed and dried with an air spray gently directed perpendicular to the gingival margin ***(Griffiths, 2003).***

Gentle removal of supra-gingival plaque was completed utilizing dry gauze, and a sterile filter paper strip ** was gently inserted into the entrance of the selected site until mild resistance was felt and left for 30 sec. Samples contaminated with blood or saliva were discarded. The strips were placed in a dry Eppendorf tube and stored at –80 °C until analysis.

Un-stimulated whole saliva was collected from all participants. Each subject was asked to abstain from drinking, eating and teeth brushing for at least 60 min preceding collection. Un-stimulated whole saliva was collected using the drooling technique. Each subject rinsed their mouth with water before saliva collection, and then the subject was asked to swallow to remove saliva from the mouth. The subject was seated upright, and leaned his/her head forward over a test tube with a funnel, allowing the saliva to drain into the tube. Whole saliva (~5 mL) was obtained from each individual. During saliva collection, the test tube was placed on ice. At the end of the collection, any remaining saliva in the patient's mouth was expelled into the test tube. unstimulated saliva was collected between 11:00 am and 13:00 pm for 5 min (one spit per minute). The saliva was collected in sterile tubes.

Saliva obtained was centrifuged at 15,000 × g *** for 15 min at 4°C to remove insoluble material. Supernatant was divided into 1-mL aliquots in pre-chilled cryo-tubes. The specimens were immediately frozen (-80°C) until analysis.

Periostin level detection by ELISA

The level of periostin was measured in saliva and GCF samples by using ELISA kit provided by *EIAab®*****. The ELISA is based on the competitive binding Enzyme Linked Immune Sorbent Assay technique. The micro-titre plate provided in this kit has been pre coated with an antibody specific to C4a, C4a in the sample or standard competes with a fixed amount of biotin-labeled C4a for sites on a pre-coated Monoclonal antibody specific to C4a. Then Avidin conjugated to Horseradish was added to each well and incubated. Then a TMB substrate solution was added. Then termination was done by the addition of sulphuric acid solution and the color change was measured spectro-photo-metrically at a wavelength of 450 nm ± 2 nm. The concentration of C4a in the samples was then determined by comparing the O.D. of the samples to the standard curve.

* The Arab Drug Company for pharmaceutical and CHEM. IND. CO. Cairo-Egypt.

** Periopaper® , Amityville, NY, USA

*** MPW-65R, MPW, Med Instrument, Warszawa, Poland

**** China Catalog No: E0638h.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.*. The Kolmogorov- Smirnov was used to verify the normality of distribution of variables; Comparisons between groups for categorical variables were assessed using Chi-square test (Monte Carlo). Student t-test was used to compare two groups for normally distributed quantitative variables; ANOVA was used for comparing the three studied groups and followed by Post Hoc test (Tukey) for pairwise comparison. Wilcoxon signed ranks test was assessed for comparison between two periods for not normally distributed quantitative variables. Kruskal Wallis test was used to compare different groups for abnormally distributed quantitative variables and followed by Post Hoc test (Dunn's for multiple comparisons test) for pairwise comparison. Significance of the obtained results was judged at the 5% level.

RESULTS

This clinical trial was conducted on a total of 60 participants. Participants were divided into three groups. Group I consisted of 20 subjects with healthy periodontium (12 males and 8 females) with mean age 30.2(± 4.9). Group II consisted of 20 no-smoker patients with stage II-III periodontitis (14 males and 6 females) with mean age 34.4 (±7.4). Group III consisted of 20 smoker patients (18 males and 2 females) with stage II-III periodontitis with mean age 33.6 (±7.6). There was no significant difference in age between the three studied groups.

Table (1) presents comparison between the three studied groups in clinical parameters and GCF and salivary periostin levels at baseline.

Clinical parameters (GI, PI, PD , CAL) were all statistically significantly lower in group I compared to groups II and III, while there was no statistically significant difference between groups II and III.

At baseline, GCF periostin level (pg/ul) was statistically significantly higher in group I than groups II and III. Group II also has statistically significant higher GCF periostin level (pg/ul) than do group III.

At baseline, salivary periostin level (pg/ul) was statistically significant lower in group I than groups II and III. Group II also has statistically significant lower salivary periostin level (pg/ul) than do group III.

Table (2) presents mean and SD values for intra-group and intergroup comparison between non-smokers and smokers in the clinical parameters before and after treatment.

In all clinical parameters (GI, PI, PPD, CAL), there was no significant difference between group II and group III either at baseline or 3 month after treatment.

However, in both groups II and III, there was a statistically significant improvement in all clinical parameters (GI, PI, PPD, CAL) 3 months after non-surgical treatment.

Table (3) presents mean and SD values for intra-group and intergroup comparison between non-smokers and smokers in GCF and salivary Periostin level before and after treatment.

At baseline as well as 3 months after treatment, GCF periostin level (pg/ul) was statistically significant higher in group II compared to Group III.

In group II as well as in group III, there was statistically significant increase in GCF periostin level (pg/ul) 3 months after treatment.

At baseline, salivary periostin level (pg/ul) was statistically significant lower in group II compared to Group III. 3 month after treatment, salivary periostin level (pg/ul) was non-significantly lower in group III compared to group II.

In group II as well as in group III, there was statistically significant reduction in salivary periostin level (pg/ul) 3 months after treatment.

Regarding percent change, group II showed

* Armonk, NY: IBM Corp

TABLE (1): Comparison between the three studied groups according to different parameters at baseline

Parameter		Healthy (n = 20)	Periodontitis		P value
			Non-smokers (n = 20)	Smokers (n = 20)	
GI	Mean ± SD.	0 ± 0	2.3[a] ± 0.7	2.3[a] ± 0.8	<0.001*
P.I	Mean ± SD.	0 ± 0	2.60[a] ± 0.52	2.70[a] ± 0.48	<0.001*
PPD	Mean ± SD.	0.50 ± 0.53	5.90[a] ± 0.88	6.40[a] ± 1.17	<0.001*
CAL	Mean ± SD.	0 ± 0	6.40[a] ± 1.78	7.20[a] ± 1.87	<0.001*
GCF Periostin (pg/ul)	Mean ± SD.	313.2 ± 10.33	106.2[a] ± 4.72	66.23[ab] ± 5.56	<0.001*
Salivary Periostin (pg/ul)	Mean ± SD.	77.8 ± 5.1	171.3[ab] ± 7.1	196.6[ab] ± 10.9	<0.001*

a: Significant with healthy group ***b: Significant with Nonsmoker group***

****: Statistically significant at p ≤ 0.05***

TABLE (2) Intra-group and intergroup comparison between non- smokers and smokers in the clinical parameters before and after treatment

	Parameter	Periodontitis		P value
		Non-smokers (n = 20)	Smokers (n = 20)	
G.I	**Before treatment** (Mean ± SD)	2.30 ± 0.67	2.30 ± 0.82	0.912
	After treatment (Mean ± SD)	0.30 ± 0.48	0.70 ± 0.82	0.353
	[z]p	0.003*	0.004*	
P.I	**Before treatment** (Mean ± SD)	2.60 ± 0.52	2.70 ± 0.48	0.739
	After treatment (Mean ± SD)	0.30 ± 0.48	0.30 ± 0.48	1.000
	[z]p	0.004*	0.004*	
PPD	**Before treatment** (Mean ± SD)	5.90 ± 0.88	6.40 ± 1.17	0.295
	After treatment (Mean ± SD)	2.90 ± 0.57	3 ± 0.67	0.722
	[t]p	<0.001*	<0.001*	
CAL	**Before treatment** (Mean ± SD)	6.40 ± 1.78	7.20 ± 1.87	0.340
	After treatment (Mean ± SD)	4.80 ± 1.32	5.40 ± 1.81	0.407
	[t]p	<0.001*	<0.001*	

****: Statistically significant at p ≤ 0.05***

statistically significant percent increase in GCF Periostin level (pg/ul) (150.7 ± 26.1) 3 months after treatment. Group III as well showed statistically significant percent increase in GCF Periostin level (pg/ul) (125.0 ± 42.4) 3 months after treatment. Group II showed statistically significant percent increase in GCF Periostin level (pg/ul) than do group III with p-value <0.001. Figure (1)

Group II showed statistically significant percent reduction in salivary Periostin level (pg/ul) (21.8 ± 8.7) 3 months after treatment. Group III as well showed statistically significant percent reduction in salivary Periostin level (pg/ul) (34 ± 6.5) 3 months after treatment. Group II showed statistically significant percent reduction in GCF Periostin level (pg/ul) than do group III with p-value <0.001. Figure (2)

TABLE (3): Intra-group and intergroup comparison between non- smokers and smokers in GCF and salivary Periostin level before and after treatment

	Parameters	Periodontitis		P value
		Non-smokers (n = 20)	**Smokers (n = 20)**	
GCF, Periostin (pg/ul)	**Before treatment** (Mean ± SD)	106.2 ± 4.72	66.23 ± 5.56	<0.001*
	After treatment (Mean ± SD)	265.8 ± 25.32	148.4 ± 26.21	<0.001*
	^{t}p	<0.001*	<0.001*	
Salivary (pg/ul)	**Before treatment** (Mean ± SD)	171.3 ± 7.1	196.6 ± 10.9	<0.001*
	After treatment (Mean ± SD)	133.5 ± 11.9	129.6 ± 12.9	0.491
	^{t}p	<0.001*	<0.001*	

: Statistically significant at p ≤ 0.05

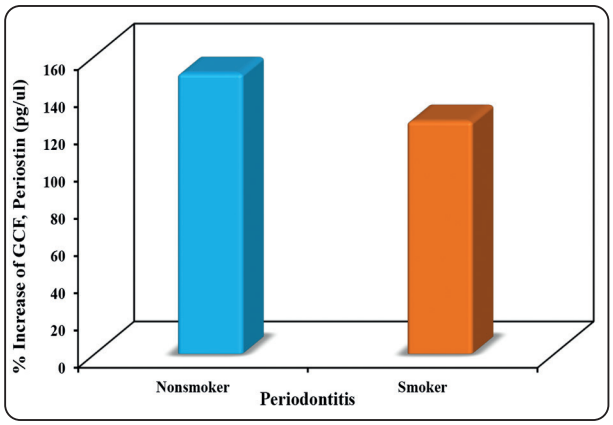

Fig. (1): Comparison between nonsmoker and smoker patients according to % increase of GCF Periostin level

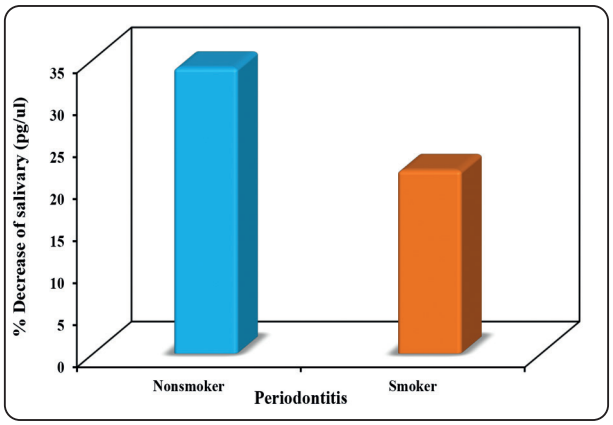

Fig. (2): Comparison between nonsmoker and smoker patients according to % reduction of salivary Periostin level

DISCUSSION

POSTN is among the most important proteins that display a vital role in the healing of wounds and periodontal defects. It serves as a modulator of hemostasis in the PDL ***(Esfahrood and Veysari, 2019).*** It is a type of cellular matrix proteins that fibroblasts secrete into the PDL and is necessary for health and maturation of tissues ***(Padial-Molina et al., 2014).*** POSTN is a protein-based biomolecule that owns a diagnostic and monitoring potency, it can be potentially utilized as a biomarker to detect periodontium physiological and pathological changes ***(Khurshid et al., 2020).***

Of the known proteins expressed in the PDL, POSTN shows the greatest specificity ***(Saito et al., 2002)***. It is localized between the cytoplasmic processes of periodontal fibroblasts and cementoblasts and the adjacent collagen fibrils. It has been, therefore, used as a marker of successful periodontal regeneration **(Suzuki *et al*., 2004)*.***

In the present study, the impact of non-surgical periodontal therapy on POSTN levels in GCF and saliva in non-smokers versus smoker patients with stage II-III periodontitis was evaluated and compared with those having healthy periodontium.

Smoking is now a well-recognized main risk factor for progression of periodontal diseases ***(Genco & Borgnakke, 2013)***. Smoking results in higher periodontitis susceptibility and less favorable response to periodontal therapy because of the adverse cigarette chemical effects on the interaction between oral micro-biota and host responses ***(Lamster, 1992)***.

To the best of authors' knowledge, previous studies have not assessed the impact of smoking on GCF or salivary level of POSTN in periodontitis. The only published research related investigated the effect of smoking on serum POSTN levels in asthmatic patients, that study declared reduced serum POSTN levels in smoking asthmatics compared to non-smoking controls ***(Thomson et al, 2015).***

The results of the present study showed a non- significant difference between non-smokers and smokers periodontitis patients in all clinical parameters (PI, GI, PPD, CAL) at baseline as well as 3 months after non-surgical therapy. whereas, non-surgical periodontal therapy led to a statistically significant reduction in all clinical parameter after 3 months in both non-smokers as well as smokers periodontitis patients.

Our results were in line with ***Gorkhali et al. (2020).*** Their study results indicated that non-surgical therapy: scaling and root planing led to a statistically significant PPD reduction and CAL gain in smokers and non-smokers with moderate periodontitis. The results showed that at baseline the clinical conditions of both groups were similar but after non-surgical therapy there was an improvement in both PPD and CAL which was found to be significant only at 1 month; however at 3 and 6 months, the difference was not significant.

Various investigations have been done for the evaluation of the effects of cigarette smoking on clinical parameters of periodontitis as well as the wound healing process following periodontal therapy ***(Gorkhali et al,. 2020).***

It is generally assumed that non-surgical periodontal therapy has an inferior clinical outcome for smokers than it does for non-smokers ***(Chang et al., 2020)***. Several studies concluded less pocket depth reduction and gain in CAL in smokers compared to their non-smokers counterparts ***(Tonetti et al., 1995; Boström et al., 1998; Heasman et al., 2006)***. On the contrary, some studies did not find such significant difference in clinical outcomes between smokers and non-smokers after non-surgical periodontal therapy ***(Preshaw et al., 2013; Feres et al., 2015; Türkoğlu et al., 2016; Guru et al., 2018).***

Guru et al.,(2018) found no significant differences in PD and CAL changes 4 weeks after full-mouth scaling and root planing between smokers and non-smokers in a prospective cohort research. Several other trials, including soft tissue grafting ***(Harris, 1994)*** and dental implant placement ***(Machtei et al., 1998),*** found no significant differences in clinical results between smokers and non-smokers following surgical periodontal therapy.

The conflict found between our results and previous studies may be attributed to that our study included both stage II and stage III periodontitis (moderate as well as deep pockets) whereas greater reduction in PD following non-surgical therapy usually takes place more in deep pockets ***(Cobb, 1996)***. Furthermore, the report of both full-mouth as well as deep site clinical outcomes specially those of PD and CAL were needed. Moreover, the impact of smoking wasn't apparent as smoking intensity and duration was not clear and these can obviously affect the clinical outcome of periodontal therapy.

GCF is an inflammatory exudate that contains a wide array of proteins, immune-inflammatory mediators and bacterial cellular elements derived from blood and periodontal tissues. GCF sample collection; being a simple, non-invasive procedure; make it an important tool monitoring periodontal disease progression as well as healing following periodontal therapy ***(Adonogianaki et al., 1992).***

Our results showed that GCF periostin level was significantly higher in healthy individuals compared to periodontitis patients. This was in line with ***Sophia et al., (2020)*** who concluded in their study that with increasing periodontal inflammation and periodontal disease severity, GCF periostin levels were decreased gradually. This study was also based on several earlier investigations which showed that the levels of GCF periostin are lower than that of healthy periodontium in chronic periodontitis ***(Balli et al., 2015; Kumaresan et al., 2016)***.

Low level of GCF periostin in periodontitis might be attributed to its role in maintaining hemostasis and integrity within normal periodontal tissues.

Nakajima et al., (2014) found that in periodontal ligament fibroblasts, P. gingivalis lipopolysaccharides and the tumor necrosis factor-α were able to down-regulate levels of POSTN, which confirms the detrimental influence of the inflammatory process on periostin levels. Consequently, the bacterial challenge of periodontal ligament fibroblasts and the related disorder in periodontitis patients would decrease GCF periostin with the resulting reduction in both structural and biochemical periodontal ligament stability potential ***(Padial-Molina et al., 2013).***

GCF periostin level was significantly lower in smoker periodontitis patients compared to non-smokers. Smoking activates inflammatory cells and cytokines ***(Wesseling, & Wouters, 2007)***, impairs neutrophil chemotaxis ***(Palmer, Watts, & Addison, 1993)*** and phagocytosis ***(Asif & Kothiwale, 2010)***, and decreases fibroblast proliferation, migration and collagen synthesis ***(Kinane & Chestnutt, 2000).***

Smoking has a well-known prolonged negative impact on both local and systemic host immune responses, including both innate and adaptive immunity ***(Mooney et al., 2001; Palmer et al., 2005)***. Furthermore, nicotine can adversely impact the reparative healing capacity by suppressing vascular proliferation, inhibiting fibroblast proliferation and adhesion, as well as collagen production ***(Tanur et al., 2000; Haffajee & Socransky, 2001).*** The aforementioned negative effects of smoking on immune response and periodontal health can justify the down-regulation of periostin GCF level in smokers compared to non-smoker patients with periodontitis.

At the 3rd month following non-surgical therapy, periostin level was increased when compared to the baseline in both group II and III. Moreover, this increase in GCF periostin level was more statistically significant in group II (non-smoker periodontitis patients) compared to group

III (smoker periodontitis patients) at the end of the study (P≤ 0.001).

The findings of current study were compatible with the study of ***Kumaresan et al., (2016)*** which investigated the effect of non-surgical periodontal therapy with adjunctive application of low level laser therapy on the level of periostin in the GCF of patients with periodontitis. It was concluded that the GCF level of periostin was lower in periodontitis than healthy subjects at baseline and its level showed statistically significant increase at 3 months following treatment. The possible explanation of these results might be due to the success of periodontal therapy in reducing the levels of pro-inflammatory cytokines in the GCF of patients with periodontitis thus confirming the role of inflammatory condition in reducing the expression of periostin in the GCF.

GCF periostin level was found to be increased in patients with chronic and aggressive periodontitis following periodontal surgery in a case-control study conducted by ***Padial-Molina et al. (2015)***. This finding further supports the pivotal role of periostin in periodontal wound healing process. In response to periodontal therapy, there was a reduction of inflammatory response and bacterial stimuli as indicated by the significant improvement in the clinical parameters which accounts for the increase in the GCF periostin level after periodontal therapy. Periostin increased to support healing process by enhancing migration and proliferation of the fibroblasts to construct a firm extracellular matrix.

Saliva is a secretion that protects and maintains health and hemostasis in the oral cavity. It has a key role on minimizing plaque accumulation by mechanically clearing exposed dental and periodontal surfaces, neutralizing acids released from bacteria and nutritional sources, and by applying an antibacterial action. Furthermore, saliva is a potent tool for the determination of periodontal disease activity and for the estimation of periodontal disease outcome ***(Salazar et al., 2013)***.

Many studies conveyed a significant association between periodontal disease severity and POSTN level ***(Padial-Molina et al., 2015; Balli et al., 2015; Kumaresan et al., 2016)***. Conversely, another study reported no significant difference in POSTN level in periodontitis patients compared to healthy controls ***(Norris et al., 2005)***.

Our study showed that salivary periostin level was statistically significantly lower in healthy individuals (group I) compared to periodontitis patients (group II and III). This was in line with ***Cüneyt et al. (2016)*** who concluded that salivary periostin levels may be associated with gingival inflammation and periodontal disease severity in the whole mouth. High salivary periostin levels in periodontitis might be caused by high serum periostin level passed into saliva by spontaneous bleeding associated with periodontal inflammation.

Padial-Molina et al. (2013) conducted an in vitro study to investigate messenger RNA expression and levels of periostin in human periodontal ligament cultures subjected to biomechanical loading and bacterial virulence factors (TNF-α and *P. gingivalis* lipopolysaccharide). Their results indicated that, under biomechanical loading and bacterial challenge, both expression and protein levels of periostin were increased in the early period of exposure, followed by a significant decrease with disease progression. The increase in periostin level might be possibly due to the continuous bacterial challenge and related immune response ***(Arslan et al., 2020)***.

Periostin is an extracellular matrix protein that plays a pivotal role in periodontal tissue healing and hemostasis through orchestring cell–matrix interactions and cell functions. This intern regulates migration, adhesion and proliferation of fibroblasts and binding of various tissues to healing sites. The migration of fibroblasts and osteoblast has a key role

in periodontal wound healing and regeneration and new bone formation ***(Du & Li, 2017).*** Moreover, ***Conway et al., (2014)*** reported that periostin level may show a transient elevation as a result of physiologic or pathologic changes within the cells.

Matrix metallopeptidases (MMPs) holds a key effect on the degradation of extracellular matrix by degrading collagen and proteoglycans of healthy and diseased subjects ***(Riley, 2008).*** POSTN promotes MMP families' expression in several cell types, thus has been observed to accelerate matrix disintegration and inflammation ***(Attur et al. 2015; Chijimatsu et al.., 2015).***

Nishiyama et al., (2011) reported delayed skin wound healing in absence of periostin as periostin was involved in inspiring keratinocyte proliferation. In line with these findings, in case of periodontal tissue damage, periostin level is expected to increase to counteract the damage and contribute to periodontal tissue repair and maturation.

Acting as both extra-cellular matrix and a matri-cellular protein, periostin have an importance in the immune-inflammatory response. As an extracellular matrix protein , periostin is incorporated into the inflamed tissues showing fibrosis, while it activates immune and non-immune cells as a matri-cellular protein, further amplifying inflammation ***(Arslan et al., 2020).*** Therefore***,*** it's not surprising to find upregulation of salivary periostin level with increase severity of periodontal disease.

Furthermore, salivary level of periostin was significantly higher in smokers compared to non-smokers. This is the first study to evaluate the level of periostin in smoker patients with periodontitis, so exact comparison with other studies isn't possible. Since smoking impairs the chemotaxis and phagocytosis of neutrophils, and harmful components of tobacco smoke may contribute to increase the production of cytokines and inflammatory mediators ***(Giannopoulou et al., 2003)***, this is correlated to the further increase in the salivary periostin level compared to non-smokers.

3 month after non-surgical periodontal therapy was associated with significant reduction in salivary periostin level in periodontitis patients with more significant reduction in group II (non-smokers) compared to group III (smokers).

According to ***Arslan et al, (2020)***, it is believed that in periodontitis at baseline, as a protective mechanism, POSTN release is up-regulated in response to bacterial challenge to afford tissue remodeling and repair. 3 month after periodontal therapy, when complete repair and maturation of periodontal connective tissue took place, the salivary periostin level declined again as in the healthy individuals.

CONCLUSION

Non-surgical therapy resulted in up-regulation of GCF POSTN level in smokers and non-smokers with significant increase in non-smokers while resulted in down-regulation of salivary periostin level in smokers and non-smokers with more significant reduction in non-smokers.

RECOMMENDATIONS

Further studies to investigate GCF and salivary POSTN levels in smoker periodontitis patients taking into consideration intensity and duration of smoking.

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