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EFFECT OF NON-SURGICAL PERIODONTAL TREATMENT ON CREVICULAR LEVELS OF CHEMERIN AND FIBROBLAST GROWTH FACTOR 21 IN CONTROLLED TYPE 2 DIABETIC AND NON-DIABETIC PATIENTS BOTH WITH PERIODONTITIS AND THEIR GLYCEMIC CONTROL. A CLINICAL TRIAL

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ABSTRACT

Background: Diabetes mellitus is one of the important host risk factors in periodontal diseases. Many factors were reported not only to play a role in the periodontal inflammation, but also to be associated with the severity of the tissue breakdown such as chemerins which are believed to be involved in a variety of chronic inflammatory conditions including diabetes. Fibroblast growth factor 21 (FGF21) is another adipokine which is connected with regulation of glucose metabolism and was shown to be increased in patients with obesity and type 2 diabetes mellitus (T2DM). The present study aimed to explore the effect of non –surgical periodontal treatment on the gingival crevicular fluid level of Chemerin and FGF21in both controlled diabetic and non-diabetic patients both suffering from periodontitis and to detect the effects of this treatment on the glycemic control of diabetic patients.

Methods: The study was conducted on 2 groups; group (A) included fifteen controlled T2 DM patients suffering from periodontitis, and group (B) included fifteen patients suffering from periodontitis.

At baseline, GCF samples were collected from all participants for assessment of Chemerin and FGF 21 levels and 3 months after scaling and root planing (SRP). Samples were analysed using ELISA technique. Blood samples were also collected for assessment of HA1c.

Results: Significantly higher levels of Chemerin and FGF21 were found in periodontitis patients with diabetes than in the periodontitis patients without diabetes. After periodontal therapy, there was a significant reduction in the GCF levels of chemerin and FGF21 in both periodontitis groups. All clinical parameters showed a significant improvement after treatment. HbA1c did not correlate significantly with any of the studied adipokines as well as any of the clinical parameters in the diabetic group.

Conclusion: Periodontitis and T2DM share the nature of inflammation, with adipokines such as Chemerin and FGF21 involved in the process of inflammation. Chemerin exerts a proinflammatory influence on periodontitis and t2DM, also, FGF21 is a potent metabolic regulator with multiple beneficial effects on periodontitis and diabetes.

KEYWORDS: Chemerin, FGF-21, HA1c, DM, periodontitis, gingival crevicular fluid

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INTRODUCTION

Periodontitis is one of the most common chronic inflammatory diseases which is characterized by destruction of the supporting structures of the teeth (Eke et al, 2015). It is characterized by gingival inflammation, bleeding on probing, as well as alveolar bone and attachment loss between the tooth and its supporting structure (Holt and Ebersole, 2005).Periodontitis is characterized by increased secretion of a variety of inflammatory mediators in the localized periodontal environment (Taiyeb-Ali et al., 2011).

Diabetes mellitus (DM) is a metabolic disorder that is characterized by hyperglycemia due to impaired insulin action. Type 2 diabetes mellitus (T2DM) is believed to be the most common form of DM as it accounts for nearly 90% of all DM patients (Afable & Karingula. 2016).DM has been found to be one of the important host risk factor in periodontal diseases in large epidemiological studies as mentioned by Loe et al, (1978). Previous studies that related periodontitis to type-2 DM, had shown increased prevalence and incidence of periodontal disease in individuals with diabetes. Moreover, Loe (1993) described periodontitis as the sixth complication of diabetes.

Periodontitis and DM were found to share a number of pathogenic features, such as increased immuno-inflammatory responses with similar biologic mediators (Duarte et al., 2014). On the same hand, the progression of DM and periodontitis comes just after mild inflammation, therefore, increased plasma and crevicular levels of pro inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor (TNF)-α, interleukin IL-6, IL-1β, and prostanoids were detected (Taiyeb-Ali et al., 2011). Similarly, proteins (adipokines) produced by the adipose tissue and the defence cells are likely to play a role in the inflammatory reaction. In addition, the adipocytes were found to generate inflammatory cytokines such as TNF-α and IL-6. Previously, the inflammatory cytokines were thought to be generated by macrophages which explain the connection between inflammation and resistance to insulin (Ogawa et al, 2014). Some adipokines were reported not only to play a role in periodontal inflammation, but also to be related proportionally with the extent of the tissue breakdown (Özcan et al, 2015)

One of these adipokines is Chemerin, which remains in its inactive form as prochemerin until under inflammatory conditions, become activated by the removal of the C-terminal amino acid by proteolytic enzymes such as cathepsin G (Bondue et al., 2011). The inflammatory effect of Chemerin was found to occur through both the induction of pro inflammatory cytokines such as IL-1β and IL-8 and stimulation of chemotaxis of the inflammatory cells to the site of inflammation (Berg et al, 2010, Yoshimura and Oppenheim, 2011,). Chemerin is also believed to be associated with a variety of chronic inflammatory conditions, such as, diabetes, rheumatoid and cardiovascular diseases (Huang et al, 2012). In a previous study, the authors determined that Chemerin, not only increases in the serum of systemic diseases, but also increased in the saliva of periodontitis patients (Özcan et al, 2015).

Fibroblast growth factor 21 (FGF21) is another adipokine that have been connected with the regulation of glucose metabolism and energy homeostasis (Simjak et al, 2018). It is one of the members of FGF superfamily that is produced predominantly by the liver and adipose tissue (Fon Tacer et al., 2010). Increased FGF21 levels were observed in patients with either obesity or type 2 diabetes mellitus (T2DM) (Dostalova et al. 2009).

There has been a steady growing trend during the past few decades to develop less invasive tools to monitor the progression and diagnosis of periodontitis. Since GCF is closely approximated to the periodontal tissues where periodontal disease starts, it provides more information than saliva

markers (Gupta, 2013). Various inflammatory markers have been detected in GCF in periodontitis individuals with and without diabetes (Pradeep et al, 2012)

Therefore, the present study aimed to explore the effect of non –surgical periodontal treatment on crevicular levels of Chemerin and FGF21 in both controlled diabetic and non-diabetic patients both suffering from periodontitis in an attempt to further understand their role in the pathogenesis that connect diabetes and periodontal disease and to detect the effect of this treatment on the glycemic control of diabetic patients.

PATIENTS AND METHODS

Thirty individuals ranging in age from 35-60 years were selected from outpatient clinic of Oral Medicine, and Periodontology Department, Faculty of Dentistry, Cairo University. The study was conducted from Mar 2018 to Dec 2018.

They were divided into the following two groups:

Group (1): 15 patients with periodontitis and controlled t2DM exclusively

Group (2): 15 patients with periodontitis and free of any other systemic diseases

Medical history was obtained according to modified Cornell medical index (Abramson, 1966). All participants were informed about the nature and objectives of the study and willingness to come for the follow up visits till the end of the study and signed an informed consent.

All patients' glycemic condition was verified using glycated hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) measures. According to the American Diabetes association 2010, patients with controlled DM, to participate in the research, had to exhibit HbA1c 6-8% and FPG ≥126 mg/dL at the start of the investigation and to verify the non-diabetic status of healthy individuals their HbA1c was <6% and FPG < 126 mg/dL.

Inclusion criteria

≥35 Age ≤ 60 years and a minimum of 20 natural teeth, not including third molars. Patients with controlled t2DM for more than one year who did not suffer from any additional systemic diseases, only t2DM.

Patients with controlled t2DM exhibiting HbA1c 6-8% and FPG ≥126 mg/dL

All diabetic patients given consistent volumes of oral antidiabetic drugs by their health care provider and had not altered their treatments within the 3 months preceding the study.

As for patients with periodontitis, participants with moderate to severe periodontitis having gingival index GI ≥ 1, probing depth PD ≥ 5, and clinical attachment level CAL ≥ 4 mm, and bone loss affecting > 30% of existing teeth on clinical/ radiographic examination according to the American academy of periodontology 2000 were included in the study.

Exclusion criteria

Periapical pathologies, exposure to mechanical force as a result of occlusion/ orthodontics, and any systemic disease such as, human immune-deficiency virus, cancer, uncontrolled diabetes mellitus, or any other diseases which may affect the biomarkers levels and the periodontal conditions, smoking over the past 5 years, exposure to steroid therapies, radiation/immune-suppressive therapies, allergic reaction to any kind of drug, etc. Participants featured no history of either periodontal or drug therapies within the preceding 6 months, such as antibiotic courses, anti-inflammatory treatments, or other pharmacological treatments.

Periodontal examination

At baseline, all participants were subjected to a full- mouth examination. For periodontitis patients, clinical parameters were recorded, including

(plaque index PI, gingival index GI, probing depth PD, and clinical attachment level CAL). Again, these parameters were recorded 3 months following scaling and root planing (SRP).

Intra-examiner reproducibility: PD and CAL were measured, and PI, GI, were recorded using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA). Individuals were assessed twice with a 48-h interval. If the difference between the two measurements fall within a < 10% mm range, then researcher measurements were considered to be acceptable (Schwarz et al, 2006).

Periodontal treatment:

Once baseline crevicular samples were taken, individuals with periodontitis were subjected to non-surgical periodontal treatment, SRP using manual scalers and curettes with local anaesthesia. Periodontal treatment was completed in 2–3 visits for 2 weeks according to each patient's requisites, and each visit was 45–60 min long. No pharmacological interventions were prescribed as part of the course of treatment; they only were given oral hygiene instructions, using the modified Bass approach, toothpaste, and interdental tools. The same researcher (W.A) performed treatment.

Samples collection and analysis:

Site Selection and GCF Collection: Clinical and radiologic examinations and sampling site selections all were conducted by a single examiner (E.A). Samples were gathered the day after patients had been given clinical assessment to avoid mixing GCF with blood associated with the probing of inflamed areas. Two sites per individual were chosen from each group. GCF samples were taken from mesio-buccal or disto-buccal sites in teeth with the highest attachment loss. Samples were then taken from these areas at baseline and 12 weeks after SRP for all periodontitis groups. Before GCF sampling, all supragingival plaque was eliminated from the sample area using a sterile cotton roll. The site was then washed with water, sectioned off with cotton balls, and gradually air-dried aiming to prevent contamination with saliva. Paper strips were positioned inside the crevice until a small amount of resistance was encountered and then allowed to remain in place for 30 seconds. This process was conducted delicately to prevent manual damage to gingival tissues. Samples consisting of either blood or saliva were disregarded. Two strips from each study participant were put into a single (coded) Eppendorf container, then combined to form one sample, and quickly stored at -80C until they could be evaluated.

Measurement of chemerin level in GCF:

The level of Chemerin was measured using the RD191136200R Human Chemerin ELISA which is a sandwich enzyme immunoassay for the quantitative measurement of human Chemerin. The kit was provided by BioVendor – Laboratorní medicína, Guang Zhou, CHINA. In the Biovendor Human Chemerin ELISA, standards, quality controls and samples are incubated in microtitration wells pre-coated with polyclonal anti-human chemerin antibody. After a 60 minute incubation followed by washing, the biotin labelled polyclonal anti-human Chemerin antibody is added and incubated with the captured Chemerin for 60 minutes. After another washing, streptavidin-HRP conjugate was added. After 30 minutes incubation and the last washing step, the remaining conjugate was allowed to react with the substrate solution (TMB). The reaction was stopped by addition of acidic solution and then the absorbance of the resulting yellow product was measured. The absorbance is proportional to the concentration of Chemerin. A standard curve then was constructed by plotting absorbance values against Chemerin concentrations of standards, and the concentrations of unknown samples are determined using this standard curve.

Quantitation of Human fibroblast growth factor 21 in GCF:

Human fibroblast growth factor 21 was measured in all participants using Human Fibroblast growth factor 21 ELISA Kit which was provided by Bioassay Technology Laboratory, Shanghai, China. This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). FGF-21 was added to the wells pre-coated with FGF-21 monoclonal antibody. After incubation, the biotin-conjugated anti-human FGF-21 antibody was added to bind to human FGF-21. After incubation, unbound biotin-conjugated anti-human FGF-21 antibody was washed away during a washing step. Streptavidin-HRP was added to bind to the biotin-conjugated anti-human FGF-21 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and colour develops in proportion to the amount of human FGF-21. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Statistical analysis:

Qualitative data were presented as frequencies (n) and percentages (%). Numerical data were presented as mean, median, standard deviation (SD), minimum and maximum values.

Statistical analysis was performed with IBM*® SPSS**® Statistics Version 20 for Windows.

RESULTS

The study was conducted on thirty individuals (12 males (40%) and 18 females (60%) ranging in age from 35-60 years with mean age 43.5 years and were divided into 2 groups.

TABLE (1) Comparing Chemerin and FGF21 (pg/ml) levels before treatment in Periodontitis patients with or without diabetes.

Variables	Diabetic patients with periodontitis		Periodontitis Alone	
	Mean (±SD)	Median (Rang)	Mean (±SD)	Median (Rang)
Chemerin	251.1 (±32.7) [a]	245 (211-312)	163 (±21.3) [b]	162 (127-201)
FGF21(pg/ml)	280 (±11.6) [a]	287 (260-299)	176.1 (±10.6) [b]	172 (158-196)

Kruskal Wallis Test was used to test the difference between the two groups in term of Chemerin, and FGF21 (pg/ml) levels and Post-hoc analysis using Mann-Whitney test was performed.

Note: Values in the same row sharing the same superscript are not significantly different at p> 0.05, while the values with different superscript are significantly different at p< 0.05.

Before treatment, regarding Chemerin and FGF21 levels, periodontitis patients with diabetes had significant higher levels of Chemerin and FGF21 than the periodontitis patients without diabetes ($p < 0.05$) (Table 1).

TABLE (2) Comparing the Chemerin, and FGF21 (pg/ml) levels After treatment in Periodontitis patients with or without diabetes.

Variables	Diabetic patients with periodontitis		Periodontitis Alone	
	Mean (±SD)	Median (Rang)	Mean (±SD)	Median (Rang)
Chemerin	160.1 (±20.1) [a]	160 (132-183)	126 (±19.9) [a]	122 (91-167)
FGF21(pg/ml)	173.3 (±108) [a]	172 (155-192)	104 (±26.1) [b]	92 (78-157)

* IBM Corporation, NY, USA.

** SPSS, Inc., an IBM Company.

Kruskal Wallis Test was used to test the difference between the two groups in term of Chemerin, and FGF21 (pg/ml) levels and Post-hoc analysis using Mann-Whitney test was performed.

Note: Values in the same row sharing the same superscript are not significantly different at p> 0.05, While the values with different superscript are significantly different at p< 0.05 in the two-sided test of equality for column means

After treatment, regarding FGF21 level, significantly higher values were observed in periodontitis patients with diabetes than periodontitis patients without diabetes. In term of Chemerin level, it did not differ significantly between the periodontitis patients with or without diabetes (table 2) .

There was a statistically significant reduction in the mean Chemerin and FGF21 after the treatment in both groups. Moreover, there was a statistically significant difference in the mean change of Chemerin (p=0.001) and FGF21 (p=0.001) levels after the treatment between the periodontitis patients with and without diabetes as seen in table 3.

TABLE (3) Comparing Chemerin and FGF21 (pg/ml) levels *before and after* treatment in Periodontitis patients with or without diabetes

Variables	Diabetic patients with periodontitis				P*	Periodontitis Alone				P**	P***
	Before		After			Before		After			
	Mean (±SD)	Median (Range)	Mean (±SD)	Median (Range)		Mean (±SD)	Median (Range)	Mean (±SD)	Median (Range)		
Chemerin	251.1 (±32.7)	245 (211- 312)	160.1 (±20.1)	160 (132-183)	**0.001**	163 (±21.3)	162 (127-201)	126 (±19.9)	122 (91-167)	**0.001**	**0.001**
FGF21(pg/ml)	280 (±11.6)	287 (260-299)	173.3 (±108)	172 (155-192)	**0.001**	176.1 (±10.6)	172 (158-196)	104 (±26.1)	92 (78-157)	**0.001**	**0.001**

**P value of Wilcoxon test comparing the Chemerin, and FGF21(pg/ml) levels in the Diabetic patients with periodontitis before and after non-surgical periodontal therapy.*

***P value of Wilcoxon test comparing the Chemerin, and FGF21(pg/ml) levels patients with periodontitis alone before and after non-surgical periodontal therapy.*

****P value of Mann-Whitney test comparing the mean change in Chemerin, and FGF21(pg/ml) levels after non-surgical periodontal therapy between the Periodontitis patients with or without diabetes.*

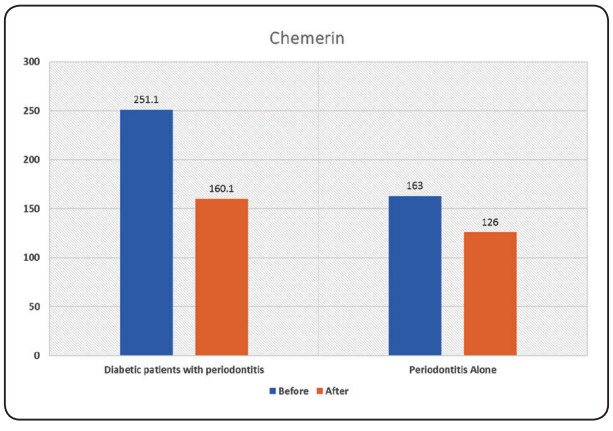

Fig. (1) Bar chart of the mean Chemerin level before and after treatment in Periodontitis patients with or without diabetes

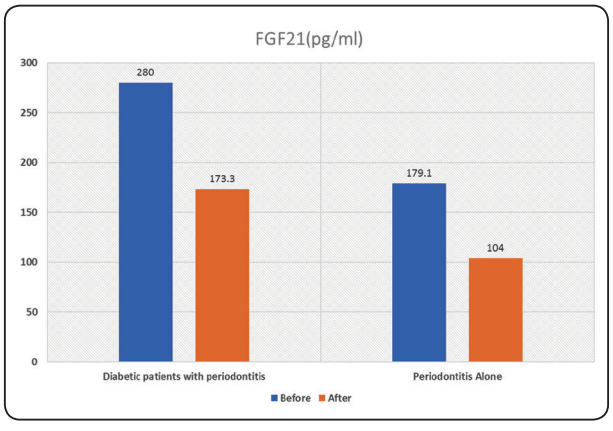

Fig. (2) Bar chart of the mean FGF21(pg/ml) level before and after treatment in Periodontitis patients with or without diabetes

TABLE (4) Correlation between Chemerin levels and HbA1c, PI, GI, PD, and CAL before and after the non-surgical periodontal therapy in the two groups.

Variables	Diabetic patients with periodontitis				Periodontitis Alone			
	Pre-operative		Post-operative		Pre-operative		Post-operative	
	Correlation	P value	Correlation	P value	Correlation	P value	Correlation	P value
HbA1c	0.132	0.640	0.198	0.479	-	-	-	-
PI	-0.185	0.509	0.142	0.615	0.041	0.884	0.233	0.403
GI	-0.340	0.214	-0.144	0.609	0.174	0.536	-0.023	0.934
PD	0.139	0.623	0.514	**0.050**	-0.431	0.108	0.284	0.304
CAL	0.364	0.182	0.112	0.691	-0.310	0.261	0.149	0.596

We observed a moderate significant positive correlation between the Chemerin level and PD post-operatively (r=0.514, P=0.05) in periodontitis patients with diabetes. In the same patients, there was no other significant correlation with the Chemerin level either pre- or post-operative as seen in table 4.

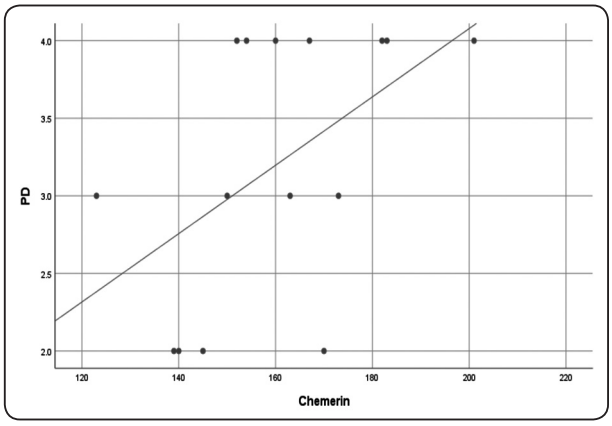

Fig. (3) Correlation between PD and Chemerin level after the non-surgical periodontal therapy in Periodontitis patients with diabetes.

TABLE (5) Correlation between FGF21 levels and HbA1c, PI, GI, PD, and CAL before and after the non-surgical periodontal therapy in the two groups

Variables	Diabetic patients with periodontitis				Periodontitis Alone			
	Pre-operative		Post-operative		Pre-operative		Post-operative	
	Correlation	P value	Correlation	P value	Correlation	P value	Correlation	P value
HbA1c	-0.527	0.044	0.158	0.573	-	-	-	-
PI	0.155	0.580	0.662	**0.007**	-0.088	0.754	-0.286	0.301
GI	0.368	0.178	-0.229	0.411	-0.309	0.263	0.393	0.148
PD	-0.087	0.759	0.032	0.910	-0.241	0.387	0.138	0.624
CAL	0.146	0.604	-0.362	0.185	0.145	0.606	0.441	0.9

FGF21 level did not correlate significantly with the HbA1c level in periodontitis patients with diabetes before and after the surgery. While a significant positive correlation was revealed between the FGF21 level and PI post-operatively (r=0.662, P=0.007) table 5.

TABLE (6) Correlation between HbA1c levels and PI, GI, PD, and CAL before and after the non-surgical periodontal therapy in the Diabetic patients.

Variables	Diabetic patients with periodontitis			
	Pre-operative		Post-operative	
	Correlation	P value	Correlation	P value
PI	-0.103	0.71	-0.12	0.68
GI	-0.23	0.41	0.03	0.92
PD	0.22	0.41	0.13	0.64
CAL	0.011	0.96	-0.37	0.17

HbA1c level did not correlate with any of the clinical parameters (PI, GI, PD, and CAL) either before or after the treatment in periodontitis patients with diabetes. Table 6

DISCUSSION

Although the association between T2DM and periodontitis has been extensively discussed, the mechanism by which periodontitis affects T2DM remains obscure (Wang et al, 2017). One of the most common and important causes of DM is dyslipidemia and an argument was raised that periodontitis may play a role in facilitation of its development within diabetics (Preshaw et al, 2012, Coimbra et al, 2014).

It has been suggested that some active molecules that is increased by periodontitis may disrupt the systematic lipid metabolism; however, the exact mechanism is a mystery. Moreover, Unhealthy adipose tissue metabolism in t2DM was believed to impact other organs, through creation of adipokines, TNF-α, IL-6, and other proinflammatory cytokines (Kim et al, 2014, Luo et al, 2016).

One of these adipokines is the Chemerin, which is recognized as a potential connection between obesity-related diseases and inflammation as mentioned by Bozaoglu et al, (2007), this can explain the result of the present study which detected a significant higher levels of Chemerin in periodontitis patients with diabetes than the non-diabetes periodontitis patients. These results are consistent with results found by Yang et al, (2010) who discovered higher Chemerin serum levels within the serum of patients with t2DM. It is also in agreement with Huang et al, (2015) who reported that Chemerin is found to be associated with a variety of chronic inflammatory conditions, such as diabetes, cardiovascular diseases and rheumatoid.

Similar to the present research, Patnaik et al, (2017) found that Chemerin levels in GCF were greater in type-2 DM with periodontitis than non-diabetic periodontitis patients. It should be mentioned that GCF is used as it offers more informative data than indicators in saliva as it is positioned very close to the periodontal tissues where periodontal disease emerges. These findings indicate that Chemerin is also generated locally within the periodontium and might represent chronic inflammation in patients with periodontitis with or without t2DM. This may suggest that it might be playing a role in the pathogenesis of periodontitis and DM. Chemerin production could also be affected by the link between t2DM and periodontitis as explained by Dogan et al, (2016).

The present study showed a significant improvement in all periodontal clinical parameters among both periodontitis groups after treatment. Our findings also detected a significant reduction of GCF Chemerin after treatment. Moreover, there was a moderate significant positive correlation between the Chemerin level and PD post-operatively in periodontitis patients with diabetes. These results are in agreement with Balli et al, (2016) who demonstrated that periodontal therapy can notably lower crevicular Chemerin level. Their results proposed that non-surgical periodontal therapy can significantly alleviate periodontal inflammation which can be partially explained by the role of chemerin as it exerts a proinflammatory influence on both periodontitis and t2DM; another explanation

is that the production of Chemerin could be provoked by raising IL-6 in patients suffering from periodontitis and t2DM symptoms.

These outcomes and their interpretation are also in agreement with Özcan et al, (2016) who detected higher salivary levels of Chemerin in periodontitis patients than in healthy individuals and its reduction 6 months after periodontal therapy. They considered Chemerin as a possible marker of inflammatory activity in the pathogenesis of both diseases; this can be attributed to the major task of Chemerin in inflammation as a chemotactic factor, where it helps cells such as macrophages and polymophonuclear leukocytes which contain ChemR23 receptors to move to the site of inflammation (Yoshimura & Oppenheim, 2011). Another role of Chemerin is triggering proinflammatory gene stimulation, such as TNF-α, IL-1β, and IL-8, in some cell types, as has been suggested by in vitro studies. (Berg et al, 2010). These preceding findings suggests that Chemerin may play a role in periodontal inflammation.

This also explains why Chemerin should be considered therapeutically valuable with respect to treatment of periodontal disease as recommended by Dogan et al, (2016). Further researches involving a greater number of participants are vital if increasing GCF Chemerin levels is to be treated as a risk-related variable for periodontal disease and t2DM.

Regarding FGF21, the present study showed significant higher levels in periodontitis patients with diabetes than the periodontitis patients without diabetes. These results are in agreement with Li et al, (2011); they detected significantly increased serum FGF21 concentrations in newly diagnosed type 2 diabetes subjects, which are similar to studies performed by Zhang et al, (2008), Chavez et al, (2009) and Mumtaz et al, (2015.). This result can be explained by the role of FGF21 as a potent metabolic regulator that carry out a vital role in glucose and lipid metabolism as suggested by Zhang et al., 2008.

These results can also be attributed to the effect of diabetes as an inflammatory condition with elevated levels of proinflammatory mediators/ markers in the serum (Freeman et al, 2002). In addition, the inflammatory response in periodontitis is characterized by the localized production of a variety of pro inflammatory mediators such as C-reactive protein (CRP), IL-1β, IL-6, TNF- α and prostanoids (prostaglandin E2). Moreover, as diabetes alters the immunologically active molecule there is an increased level of cytokines detected in the periodontal tissues which accelerates the disease progression, providing the scientific basis for the increased susceptibility of the individual to periodontal disease seen in diabetes (Mealey et al, 2007).

Up to the authors' knowledge, the present study is the first to focus the light on the effect of periodontal therapy on the GCF level of FGF21. In the current study, a significant reduction in the mean level of FGF21 after the treatment in periodontitis patients with and without diabetes was observed. However, even after treatment, FGF21 level showed a significantly higher values in the diabetic group than the non-diabetic periodontitis group. Conversely, a study performed by Wang et al, (2017) found that the serum FGF21 levels was significantly increased after therapy, which indicated that the effective control of inflammation by periodontal therapy may contribute to increasing systemic insulin-sensitizing adipokines, such as FGF21, thus in turn improving the overall health status. This discrepancy might be explained that, in the current study, the increase in crevicular FGF21 before treatment might act as a protective mechanism to oppose periodontal inflammation and as the inflammation subsided after treatment, the need for such protection is receded.

Another explanation, is that as mentioned before, no previous studies were carried on to observe the crevicular level of FGF21 changes, where, although GCF is representative for the serum, however, the periodontal environment is unique and so changes of crevicular levels of different biomarkers may be due to this special locality. Therefore, additional

investigations with larger sample size and longer – follow up period are recommended.

On the same hand, FGF21 act as a stress-responsive factor as its activation is induced by different stressful stimuli, such as hypoxia, oxidative stress, inflammation, and glucose or amino acids deprivation aiming to maintain tissue homeostasis. FGF21 was found to share in protective autocrine/paracrine loops leading to cellular stress resistance, and activation of anti-oxidation and inflammation reduction mechanisms (Salminen et al., 2017)

Likewise, Li et al (2018) also showed that FGF21 has a potential role in anti-inflammation and immunoregulation. Moreover, treatment with exogenous FGF-21 can alleviate LPS-induced inflammation and that the mechanism of action of FGF-21 was observed to involve the elevation of IL-10. Their investigation obviously pointed out that FGF21 can be utilized as an attractive goal for managing inflammatory conditions.

Regarding HbA1c level, although it reflects the glycemic level over the previous 3 months; the present study found that HbA1c level did not correlate with any of the clinical parameters (PI, GI, PD, and CAL) either before or after the treatment in periodontitis patients with diabetes. This result is similar to the multicentre, randomized clinical trial that reported that, at 6 months, the mean HbA1c level in the periodontal therapy group increased about 0.17% compared with 0.11% in the control group with no significant difference between the two groups (Engebretson et al, 2013).

Several outcomes of other meta-analyses also did not support the notion that periodontal treatment lowers the level of HbA1cas reported by Gay et al, (2014) and Simpson et al, (2015). On the other hand, Teshome and Yitayeh, (2016) performed a meta-analysis, and the results revealed a statistically significant HbA1c reduction of 0.48 (95% CI: 0.18, 0.78) in the treatment group when compared with the control group. Similarly, Wang et al, (2017) showed that the intervention group showed a significantly greater change in the HbA1c level, which indicated that periodontal treatment, may improve the glycemic control. Accordingly, whether periodontal therapy reduces the HbA1c level in periodontitis patients still remain controversial.

Moreover, the moderate correlation of these adipokines with clinical parameters may put forward the idea that reducing their levels or inhibiting their increment that may in turn prevent the progression of periodontal disease. In addition, management of adipocytokines levels may help improvement of preventive, diagnostic, and therapeutic strategies against the periodontitis as recommended by Balli et al, (2016)

Finally, larger and long-term studies are recommended to be conducted in the future. As the small sample size included in the present study represent one of the limitations to this study. Another limitation is the small period of follow up, while the long-term effects of intervention should be put in mind.

CONCLUSION

Chemerin exerts a proinflammatory influence on periodontitis and t2DM, also. FGF21 is strongly associated with diabetes and might be involved in an anti-inflammatory role in the periodontal disease process yet this role is still unclear and needs further investigations either to augment or to eliminate the notion.

REFERENCES

- Abramson JH. (1966)The Cornell Medical Index as an epidemiological tool. Am J Public Health N; 56:287-298.
- Afable A and Karingula NS. (2016). Evidence based review of type 2 diabetes prevention and management in low and middle income countries. World J Diabetes.;7(10):209-29..
- American Academy of Periodontology (2000). Parameter on chronic periodontitis with slight to moderate loss of periodontal support. J Periodontol; 71:853–855.
- American Diabetes Association 2010. Diagnosis and classification of diabetes mellitus. Diabetes Care; 33 Suppl 1:S62-9.

- Balli U , Ongoz F Dede , Bozkurt S Dogan , Gulsoy Z, Sertoglu E. (2016). Chemerin and interleukin-6 levels in obese individuals following periodontal treatment. Oral Diseases 22, 673–680.
- Berg V, Sveinbjo¨rnsson B, Bendiksen S, Brox J, Meknas K, Figenschau Y. (2010). Human articular chondrocytes express ChemR23 and chemerin; ChemR23 promotes inflammatory signalling upon binding the ligand chemerin (21-157). Arthritis Res Ther 12: R228.
- Bondue B, Wittamer V, Parmentier M. (2011). Chemerin and its receptors in leukocyte trafficking, inflammation and metabolism. Cytokine Growth Factor Rev; 22: 331-338.
- Bozaoglu K, Bolton K, McMillan J, et al. (2007). Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology 148:4687-4694.
- Chavez AO, Folli F, Molina-Carrion M, Defronzo R, AbdulGhani MA, Tripathy D. (2009). Circulating fibroblast growth factor21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. Diabetes Care 32:1542–6)
- Coimbra S, Brandao Proenca J, Santos-Silva A, Neuparth MJ. (2014). Adiponectin, leptin, and chemerin in elderly patients with type 2 diabetes mellitus: A close linkage with obesity and length of the disease. Biomed Res Int 2014: 701915.
- Dogan SB, Balli U, Dede FO, Sertoglu E, Tazegul K (2016). Chemerin as a novel crevicular fluid marker of periodontitis patients with type 2 diabetes mellitus. J Periodontol 10.1902.
- Dostalova I, Haluzikova D, Haluzik M. (2009). Fibroblast growth factor 21: a novel metabolic regulator with potential therapeutic properties in obesity/type 2 diabetes mellitus. Physiol Res 58: 1-7.
- Duarte PM, Bezerra JP, Miranda TS, Feres M, Chambrone L, Shaddox LM. (2014). Local levels of inflammatory mediators in uncontrolled type 2 diabetic subjects with chronic periodontitis. J Clin Periodontol 41:11-18.
- Eke PI, Dye BA, Wei L, Slade GD, Thornton-Evans GO, Borgnakke WS et al (2015). Update on prevalence of periodontitis in adults in the United States: NHANES to 2012. J Periodontol. 86(5):611-22.
- Engebretson SP, Hyman LG, Michalowicz BS, Schoenfeld ER, Gelato MC, Hou W et al. (2013). The effect of nonsurgical periodontal therapy on hemoglobin A1c levels in persons with type 2 diabetes and chronic periodontitis: a randomized clinical trial. JAMA. 310(23):2523-32.)
- Fon Tacer K, Bookout AL, Ding X, Kurosu H, John GB, Wang L, Goetz R, Mohammadi M, Kuro-O M, Mangel SDORF DJ, Kliewer SA: (2010). Research resource: comprehensive expression atlas of the fibroblast growth factor system in adult mouse. Mol Endocrinol 24: 2050-2064.
- Freeman DJ, Norrie J, Caslake MJ, Gaw A. (2002). West of Scotland Coronary Prevention Study Group C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention study. Diabetes 51: 1596–600.
- Gay IC, Tran DT, Cavender AC, Weltman R, Chang J, Luckenbach E et al. (2014). The effect of periodontal therapy on glycaemic control in a Hispanic population with type 2 diabetes: a randomized controlled trial. J Clin Periodontol. 41(7):673-80.
- Gupta G. (2013). Gingival crevicular fluid as a periodontal diagnostic indicator – II: inflammatory mediators, host-response modifiers and chair side diagnostic aids. J Med Life; 6: 7–13.
- Holt SC, Ebersole JL. (2005). Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia: the "red complex", a prototype polybacterial pathogenic consortium in periodontitis. Periodontol 2000; 38: 72–122.
- Huang K, Chen A, Zhang X, et al. (2015). Progranulin is preferentially expressed in patients with psoriasis vulgaris and protects mice from psoriasis-like skin inflammation. Immunology 145:279-287.
- Huang K, Du G, Li L, Liang H, Zhang B. (2012). Association of chemerin levels in synovial fluid with the severity of knee osteoarthritis. Biomarkers. 17(1):16–20.
- Kim SH, Lee SH, Ahn KY, et al. (2014). Effect of lifestyle modification on serum chemerin concentration and its association with insulin sensitivity in overweight and obese adults with type 2 diabetes. Clin Endocrinol (Oxf) 80:825-833.
- Li X, Fan X, Ren F, Zhang Y, Shen C, Ren G, Sun J, Zhang N, Wang W, Ning G, Yang J. (2011): Serum FGF21 levels are increased in newly diagnosed type 2 diabetes with non alcoholic fatty liver disease and associated with hsCRP levels independently. Diabetes research and clinical practice 93(1):10-6.
- Li JY, Wang N, Khoso MH, Shen CB, Guo MZ, Pang XX, Li DS, Wang WF. (2018). FGF-21 Elevated IL-10 Production to Correct LPS- Induced Inflammation. Jun 41(3):751-759.

- Loe H, Anerud A, Boysen H, Smith ∈ M. (1978). The natural history of periodontal disease in man. The rate of periodontal destruction before 40 years of age. J Periodontol 49: 607–20.
- Loe H. (1993). Periodontal disease. The sixth ∈ complication of diabetes mellitus. Diabetes Care; 16: 329–34.
- Luo S, Yang X, Wang D, et al. (2016). Periodontitis contributes to aberrant metabolism in type 2 diabetes mellitus rats by stimulating the expression of adipokines . J Periodontal Res. 51(4):453-61
- Mealey BL, Ocampo GL. (2007). Diabetes mellitus and periodontal disease. Periodontol 2000; 44: 127–53. 12
- Mumtaz S, Alsaif S, Wray S, Noble K. (2015). Inhibitory effect of visfatin and leptin on human and rat myometrial contractility. Life Sci 125: 57-62.
- Özcan E, Saygun NI, Serdar MA, Kurt N. (2015). Evaluation of the salivary levels of visfatin, chemerin, and progranulin in periodontal inflammation. Clin Oral Investig; 19:921-928.
- Özcan E, Işıl Saygun N, Serdar MA, Umut Bengi V, Kantarcı A (2016). Non-Surgical Periodontal Therapy Reduces Saliva Adipokine and Matrix Metalloproteinase Levels in Periodontitis. J Periodontol 87:934-943.
- Ogawa H, Damrongrungruang T, Hori S, et al (2014). Effect of periodontal treatment on adipokines in type 2 diabetes. World J Diabetes 5:924-931.
- Patnaik K, Pradeep AR, Nagpal K, Karvekar S, Singh P, Raju A. (2017). Human chemerin correlation in gingival crevicular fluid and tear fluid as markers of inflammation in chronic periodontitis and type-2 diabetes mellitus. J Investig Clin Dent 8(1).
- Pradeep AR, Priyanka N, Prasad MV, Kalra N, Kumari M. (2012). Association of progranulin and high sensitivity CRP concentrations in gingival crevicular fluid and serum in chronic periodontitis subjects with and without obesity. Dis Markers. 33:207–13.
- Preshaw PM, Alba AL, Herrera D, et al. (2012). Periodontitis and diabetes: A two-way relationship. Diabetologia 55:21-31.
- Salminen A, Kaarniranta K, Kauppinen A (2017). Integrated stress response stimulates FGF21 expression: systemic enhancer of longevity. Cell Signal 40:10–21.
- Schwarz F, Bieling K, Latz T, Nuesry E, Becker J (2006). Healing of intrabony peri-implantitis defects following application of a nanocrystalline hydroxyapatite (Ostim) or a bovine-derived xenograft (Bio-Oss) in combination with a collagen membrane (Bio-Gide). A case series. J Clin Periodontol 33: 491–499.
- Šimjaki, Cinkajzlova A., Anderlova K., Kloukova J., Kratochvilova H, et al. (2018). Changes in Plasma Concentrations and mRNA Expression of Hepatokines Fetuin A, Fetuin B and FGF21 in Physiological Pregnancy and Gestational Diabetes Mellitus Physiol. Res. 67 (Suppl. 3): S531-S542.
- Simpson TC, Weldon JC, Worthington HV, Needleman I, Wild SH, Moles DR et al. (2015). Treatment of periodontal disease for glycaemic control in people with diabetes mellitus. Cochrane Database Syst Rev. 6(11):CD004714.
- Taiyeb-Ali TB, Raman RP, Vaithilingam RD. (2011) Relationship between periodontal disease and diabetes mellitus: an Asian perspective. Periodontol 2000; 56: 258–68..
- Teshome A and Yitayeh A. (2016). The effect of periodontal therapy on glycemic control and fasting plasma glucose level in type 2 diabetic patients: systematic review and meta-analysis. BMC Oral Health. 17(1):31-42.
- Wang S, Liu J, Zhang J, Lin J, Yang S, Yao J, Du M. (2017). Glycemic control and adipokines after periodontal therapy in patients with Type 2 diabetes and chronic periodontitis. Braz. Oral Res.;31:e90.
- Yang M, Yang G, Dong J, et al. (2010). Elevated plasma levels of chemerin in newly diagnosed type 2 diabetes mellitus with hypertension. J Investig Med 58: 883-886.
- Yoshimura T and Oppenheim JJ. (2011). Chemokine-like receptor 1 (CMKLR1) and chemokine (C-C motif) receptor-like 2 (CCRL2); Two multifunctional receptors with unusual properties. Exp Cell Res 317:674-684.
- Zhang X, Yeung DCY, Karpisek M, Stejskal D, Zhou ZG, Liu F, et al. (2008). Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes 57:1246–53.