Original Article

Possible role of gingival crevicular fluid levels of Chemerin and Fibroblast growth factor 21 as biomarkers of periodontal disease in diabetic and non-diabetic patients. A diagnostic accuracy study

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Abstract

Background: The relation between diabetes mellitus and periodontitis has been discussed for years. Many adipokines were reported to play a major role in periodontal inflammation. Chemerin is one of these adipokines which is blamed to be involved in inflammatory conditions such as diabetes. Fibroblast growth factor 21 (FGF21) is another adipokine which is believed to induce regulation of glucose. The current study aimed to spot the light on the potential role of gingival crevicular fluid (GCF) level of FGF21 and chemerin as biomarkers of periodontal disease activity and an attempt to understand their role in the link between periodontitis and diabetes. **Methods:** The study was conducted on 45 individuals, 15 diabetic patients with periodontitis, 15 periodontitis patients and 15 healthy controls. GCF samples were collected from all participants for assessment of chemerin and FGF 21. Samples were analysed using enzyme linked immunosorbent assay (ELISA). **Results:** Periodontitis patients with diabetes had significant higher levels of chemerin and FGF21 than the periodontitis patients without diabetes followed by healthy controls. ROC analysis showed a 100% diagnostic accuracy for chemerin and FGF21. **Conclusion:** FGF21 and chemerin in GCF are valuable biomarkers of periodontal disease suggesting a role in the link between diabetes and periodontitis.

Keywords: Chemerin; FGF-21; DM; periodontitis; gingival crevicular fluid

1. Introduction

Periodontitis is an extensive damaging inflammatory condition of the anatomy surrounding and supporting the teeth, that is initiated due to the interruption of normal homeostasis by abundant bacterial classes existing in subgingival dental plaque (Darveau, 2010), and is altered by genetic and environmental factors(Darveau, 2010, Pihlstrom et al., 2005). This leads to tissue injury involving loss of connective tissue attachments and alveolar bone damage, frequently resulting in compromised mastication and ultimately tooth loss (Pihlstrom et al., 2005)

Periodontitis is characterized by a variety of cytokines, chemokines, arachidonic acid metabolites, and inflammatory mediators including proteolytic enzymes which are released as a response to the activities of the tissue defense systems against the microbiological agents. These released molecules were blamed for soft tissue degradation and bone resorption in the host tissue (Lindberg-Yücel and Bage, 2013).

Diabetes mellitus (DM) is a cluster of metabolic disorders that are chiefly characterized by hyperglycemia due to impaired secretion or action of insulin, or both. The mainstream of cases of diabetes falls into two wide etiopathogenetic classes. Type 1 diabetes, which comprises only 5–10% of those having diabetes, the cause of which is an absolute deficiency of insulin secretion due to an autoimmune process. The most common form of diabetes is type 2 diabetes mellitus (T2DM), which accounts for nearly 90-95% of all diabetic patients and is due to a combination of insulin resistance and an insufficient compensatory insulin secretion response (American Diabetes Association, 2013).

For decades, a significant close association between diabetes and periodontitis has been confirmed, where evidence suggests that diabetes is a contributing factor in worsening the condition of periodontal disease, on the same hand, periodontal disease has a higher incidence in diabetics, and is claimed to be more prevalent and severe in diabetics when compared to healthy individuals (Grossi and Genco, 1998, Papapanou, 1996).

Coexistence of periodontitis in diabetic patients is attributed to failure of the body's immune system to completely eliminate the source of inflammation such as microorganisms, this in turn, keeps the inflammatory process continuously activated, therefore, a chronic inflammatory reaction is induced(Herring and Shah, 2006). This chronic inflammatory response is the main etiologic factor of systemic upregulation of different pro-inflammatory cytokines such as interleukins (IL-1β, 4, 6, 8, and 10) as well as tumor necrosis factor-α (TNF-α) (Grossi and Genco, 1998, Iacopino, 2001, Sun et al., 2010, Correa et al, 2010).

Moreover, these pro-inflammatory cytokines are blamed to be a great player in inducing insulin resistance, initiating pancreatic beta-cell destruction, and dis-regulating lipid metabolism, leading to hyperlipidemia with both low-density lipoproteins and triglycerides (Iacopino, 2001, Sun et al., 2010).

Adipokines are cytokines not only derived from adipocytes but also from endothelial cells, fibroblasts and various amounts of immune cells as macrophages, lymphocytes, granulocytes, mast cells. Adipokines regulate important biological processes in target organs such as the brain, liver, skeletal muscle, cardiovascular and immune systems (Feve et al.,2016) Insulin resistance is associated with an excessive metabolic abnormalities including dyslipidemia, low grade inflammation and altered adipokine levels and many of these abnormalities can directly or indirectly accelerate the malfunctioning of glucose metabolism (Kumari et al., 2019).

Chemerin is one of the adipokines, which was first discovered as a chemoattractant peptide that believed to cause migration of macrophages, leukocytes, and dendritic cells all with ChemR23 receptors to the site of inflammation. (Monnier et al., 2018). Although the white adipose tissue is described as the main source of chemerin, later on, a high concentration was also identified in the liver (Döcke et al., 2013 and Weigert et al., 2010), lower lungs, brown adipose tissue, heart, ovaries, kidneys, skeletal muscle, and pancreas (Rourke et al., 2013).

It was demonstrated that chemerin is mostly associated with a variety of chronic inflammatory conditions, such as diabetes, rheumatoid arthritis and cardiovascular diseases (Huang et al., 2015). In addition, circulating chemerin levels was also correlated positively with TNF-α, IL-6 and C-reactive protein (CRP), resistin and leptin which are suggested as markers of inflammation. (Lehrke M et al, 2009, Weigert et al., 2010) Therefore, systemic chemerin is mainly related to inflammation rather than obesity in type 2 diabetes. Consequently, Chemerin is associated with markers of inflammation and components of the metabolic syndrome (Lehrke et al., 2009).

Fibroblast growth factor 21 (FGF21) is another adipokine which is a 181 amino acid (w20 kDa) circulating protein that is derived from a 209-amino acid mature protein encoded by the FGF21 gene which is located in chromosome 19 (Nishimura et al., 2000), FGF21 is found to belong to the human FGF superfamily, which was initially named according to its ability to stimulate fibroblast proliferation (Long and Kharitonenkov, 2011)

FGF21 is found to be expressed in several tissues such as liver (Nishimura et al., 2000), pancreas (Johnson et al., 2009), and adipocytes (Zhang et al., 2008). It is demonstrated to act as a metabolic regulator as it applies a variety of pharmacological effects on metabolism (Itoh, 2014). Moreover, circulating FGF21 levels are found to be elevated in various metabolic diseases, such as obesity, insulin resistance, and type 2 diabetes mellitus (Zhang et al., 2008, Mraz et al., 2009)

FGF21 is blamed to play various actions in energy metabolism and injury protection as a hepatokine, adipokine, and myokine in an endocrine or autocrine/paracrine manner (Itoh, 2014). Although of the several metabolic effects of FGF2, whether its action is beneficial or detrimental in the physiological or pathological conditions are still unclear (Tezze et al., 2019).

Various inflammatory markers in GCF have been detected to play a significant role in the disease mechanism of both periodontitis and type 2 DM (Pradeep et al., 2013). Therefore, the present study aimed to spot the light on the possible role of crevicular levels of both FGF21 and chemerin as potential biomarkers of periodontitis in diabetic and non-diabetic patients in an effort to understand their role in the link between both diseases.

2. Materials and Methods

This clinical trial has been registered at Clinical Trials.gov (identifier NCT04012983) and has been approved by the research ethics committee of the faculty of Dentistry, Cairo University by the number 19632 in 25-6-2019.

The present study was conducted on forty five individuals (ranging in age from 35-60 years) recruited from the outpatient clinic of Oral Medicine, and Periodontology Department, Faculty of Dentistry, Cairo University in the period from Mar 2018 to September 2018.

The participants were divided into the following three groups:

Group (1): 15 patients with periodontitis and controlled t2DM and no other systemic disease.

Group (2): 15 systemically healthy individuals having periodontitis.

Group (3): 15 periodontally and systemically healthy individuals.

Medical history for all participants was acquired according to the modified Cornell medical index (Abramson, 1966), and an informed consent was signed by all participants after explaining the nature and objectives of the study.

Glycated hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) measures were used to verify all patients' glycemic condition. At the start of the investigation, patients with DM eligible to participate in the study were determined according to the American Diabetes Association 2010, and had to exhibit HbA1c value of 6-8% and FPG ≥126 mg/dL. In order to verify the non-diabetic status of healthy individuals their HbA1c was shown to be <6% and FPG < 126 mg/dL.

Inclusion criteria

All participants were selected with age ranging between 35 and 60 years with a minimum of 20 natural teeth. Controlled type 2DM patients exhibiting HbA1c 6-8% and FPG ≥126 mg/dL for more than one year who did not complain of any systemic diseases other than t2DM.

As for periodontitis patients in groups 1 and 2, and according to the American academy of periodontology 2000, 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 were included in the present investigation.

Exclusion criteria

Systemic diseases such as, uncontrolled diabetes mellitus, human immune-deficiency virus, cancer, or any other diseases which may affect the biomarkers levels and the periodontal conditions. Exposure to steroid therapies, radiation/immune-suppressive therapies, allergic reaction to any kind of drug. Smoking over the past 5 years.

Periapical pathologies, exposure to mechanical force as a result of occlusion/ orthodontics. History of either periodontal or drug therapies within the previous 6 months, as antibiotic therapy, anti-inflammatory drugs, or other pharmacological treatments.

Periodontal examination:

All participants were subjected to a full- mouth examination. As for periodontitis patients, clinical parameters were recorded, including plaque index (PI), gingival index (GI), probing depth (PD), and clinical attachment level (CAL). PD was measured from the gingival margin to the base of the periodontal pocket, and CAL was measured from the cemento-enamel junction to the base of the periodontal pocket. All of these readings were recorded at six sites (mesio-buccal, mesio-lingual, mid-buccal, disto-buccal, distolingual, and mid-lingual) for all teeth.

Clinical parameters were recorded using a Williams periodontal probe (Hu-Friedy, Chicago, IL, USA) by the same researcher (EA). To ensure intra-examiner reproducibility, individuals were evaluated twice with a 48-h interval. A difference falling within a < 10% mm range between the two measurements, was considered to be acceptable (Schwarz et al., 2006).

GCF Samples collection:

For all groups, samples were assembled the day after the patients had been given clinical evaluation to evade mixing GCF with blood accompanying probing of inflamed areas. For each individual in group 1 and 2, two sites were chosen for sampling. GCF samples were obtained from mesio-buccal or disto-buccal sites in teeth with the most severe attachment loss. Before GCF sampling, supra-gingival plaque was removed from the sampling site by means of a sterile cotton roll. The site was then washed with water, secluded with cotton balls, and slowly air-dried to avoid saliva contamination. Paper strips were then located inside the crevice until a small amount of resistance was met and then remained in place for 30 seconds. This procedure was performed carefully to prevent damage to gingival tissues manually. Any blood and/or saliva contaminated samples were discarded. As for the healthy control group, samples were acquired from the upper first molar. From each study participant, two strips were put into a single (coded) Eppendorf container, then combined to form a single 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. Following 30 minutes incubation and the final washing step, the remaining conjugate was allowed to react with the substrate solution (TMB). Acidic solution was then added to stop the reaction 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. Using this standard curve, the concentrations of unknown samples are determined.

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.

Sample size calculation:

Sample size calculation was done based on the previous papers by Wang et al.,2017 and Šimják et al.,2018; that reported the difference in FGF-21 level between diabetic patients with periodontitis and healthy controls to be 100±70 pg/mL. 14 patients were needed to detect that difference using power 95% and 5% significance level. Sample size calculation was achieved using PS: Power and Sample Size Calculation software Version 3.1.2 (Vanderbilt University, Nashville, Tennessee, USA).

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. Kruskal Wallis Test was used to test the difference between the three groups in term of Chemerin (ng/ml), and FGF21 (pg/ml) levels and Post-hoc analysis using Mann-Whitney test was performed. Diagnostic performance of different markers was done by Roc curve. All p-values are two-sided. P-values < 0.05 were considered significant

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

3.Results

The study was conducted on forty five individuals (22 males (48.9%) and 23 females (51.1%) ranging in age from 35-60 years with mean age 43.5 years and were divided into 3groups

In terms of the clinical parameters in periodontitis patients with or without diabetes, there was no significant difference in all clinical parameters PI, GI, PD, CAL, between the two groups with P values, 0.6766, 0.3122, 0.4376, and 0.2480 respectively. ($p < 0.05$)

Regarding chemerin and FGF21 levels, periodontitis patients with diabetes had a significantly higher levels of chemerin and FGF21 than the periodontitis patients without diabetes, both being significantly higher than and healthy controls as well ($p < 0.05$) (table 1)

Concerning the correlation between HbA1c levels and PI, GI, PD, and CAL in diabetic patients with periodontitis, HbA1c level did not correlate with any of the clinical parameters (PI, GI, PD, and CAL) where (r= -0.103, p=0.71), (r=-0.23, p=0.41), (r=0.22, p=0.41), (r=0.011, p=0.96) respectively. ($p < 0.05$)

As for the correlation between chemerin levels and each of HbA1c, PI, GI, PD, and CAL in both groups, in periodontitis patients with diabetes, positive correlations were observed with the HbA1c (r=0.132, p=0.640), PD (r=0.139, p=0.623) and CAL (r=0.364, p=0.182) which were not significant, whereas negative but also not significant correlations were observed with PI (r=-0.185, p=0.509) and GI (r=-0.340, p=0.214). As for periodontitis patients alone, negative but not significant correlations between chemerin level and both PD (r=-0.431, p=0.108) and CAL (r=-0.310, p=0.261), while positive and also not significant correlations were observed between chemerin level and both PI (r=0.041, p=0.884) and GI (r=0.174, p=0.536). ($p < 0.05$)

Regarding the correlation between FGF21 levels and each of HbA1c, PI, GI, PD, and CAL in both groups. Negative but not significant correlations were observed with HbA1c (r=-0.527, p=0.044) and PD (r=-0.087, p=0.759) in periodontitis patients with diabetes. Other positive correlations were observed with the PI (r=0.155, p=0.580), GI (r=0.368, p=0.178) and CAL (r=0.146, p=0.604) which were also not significant. As for periodontitis patients alone, negative but not significant correlations between FGF21 level and PI (-0.088, p=0.754), GI (r=-0.309, p=0.263) and PD (-0.241, p=0.387) while positive but not significant correlation was observed between the FGF21 level and CAL (r= 0.145, p=0.606). ($p < 0.05$)

ROC curve analysis was implemented in this study to determine the diagnostic accuracy of chemerin

Variables	Diabetic patients with periodontitis		Periodontitis Alone		Healthy Control	
	Mean (±SD)	Median (Rang)	Mean (±SD)	Median (Rang)	Mean (±SD)	Median (Rang)
Chemerin (ng/ml)	251.1 (±32.7) [a]	245 (211- 312)	163 (±21.3) [b]	162 (127-201)	89(±8) [c]	89 (71-105)
FGF21(pg/ml)	280 (±11.6) [a]	287 (260-299)	176.1 (±10.6) [b]	172 (158-196)	81(±12) [c]	87 (60-99)

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.

Table 1: Comparing chemerin and FGF21 levels in Periodontitis patients with or without diabetes and Healthy control.

Biomarker	Cut off point	Sensitivity %	Specificity %	AUC (95% CI)	*P value*
Chemerin (ng/ml)	> 105	100	100	1 (0.921 to 1.000)	< 0.0001
FGF21 (pg/ml)	> 99	100	100	1 (0.921 to 1.000)	< 0.0001

Table 2: ROC curve interpretation of chemerin and FGF21 in GCF for differentiating periodontitis patients (diabetic and non-diabetic) from healthy controls.

Biomarker	Cut off point	Sensitivity %	Specificity %	AUC (95% CI)	*P value*
Chemerin (ng/ml)	> 201	100	100	1 (0.884 to 1.000)	< 0.0001
FGF21 (pg/ml)	> 196	100	100	1 (0.884 to 1.000)	< 0.0001

Table 3: ROC curve interpretation of chemerin and FGF21 in GCF for differentiating periodontitis with diabetes from periodontitis alone.
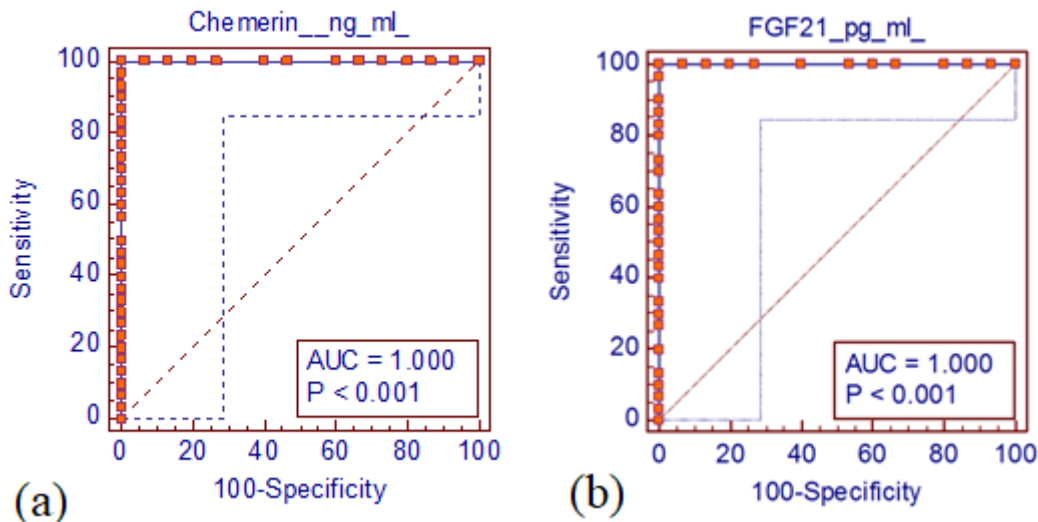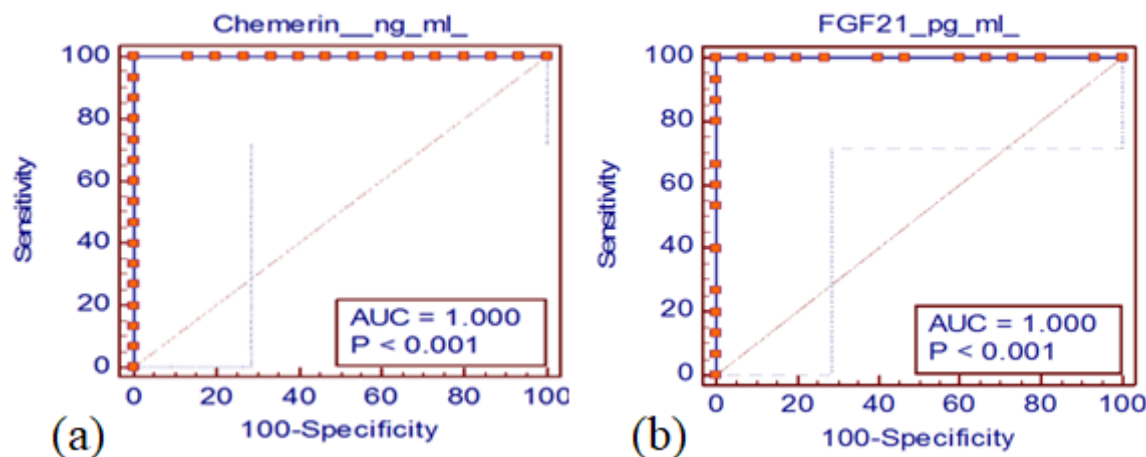

Fig.1: ROC curve for **(a)** Chemerin and **(b)** FGF21 in GCF for distinguishing periodontitis from healthy periodontium.

Fig.2: ROC curve for GCF **(a)** chemerin and **(b)** FGF21 for distinguishing periodontitis with diabetes from periodontitis alone.

and FGF21 for the differentiation between patients with periodontitis and healthy controls. This is presented in table (2) and (fig. 1). Analysis of the ROC curve revealed that for both chemerin and FGF21 the AUC was highest (1) with a 100% sensitivity and specificity. The cut-off values beyond which the individual is said to have periodontitis were decided at > 105 ng/ml and > 99 p/ml for chemerin and FGF21 respectively.

In order to distinct between individuals with periodontitis alone and those with periodontits and diabetes, ROC curve interpretation showed the highest AUC of 1 with a 100% sensitivity and specificity for both chemerin and FGF21. The cut-off value was determined at > 196 pg/ml for FGF21 and > 201 ng/ml for chemerin with a 100% diagnostic accuracy at this value. This means that subjects with GCF level of FGF21 and chemerin exceeding these cut-off values are considered to have periodontitis and diabetes while those showing a reading below this level are considered to have periodontitis alone. Table (3) and (fig. 2)

4.Discussion

Unhealthy adipose tissue metabolism detected in t2DM was believed to adversely impact other organs including periodontal tissues, through creation of a variety of biomarkers such as, adipokines, TNF-α, IL-6, as well as other pro-inflammatory cytokines (Kim et al., 2014, Luo et al., 2016).

Chemerin is one of these adipokines that was described as a concealed connection between obesity-related diseases and inflammation as reported by Bozaoglu et al., (2007). It triggers proinflammatory gene stimulation, such as TNF-α, IL-1β, and IL-8, in a variety of cell types, as explored previously by the in vitro studies (Berg et al., 2010). Therefore, chemerin is blamed for regulating the inflammatory process which is also attributed to its role in promoting the migration of macrophages to certain tissues (Goralski et al., 2007).

In the present study, GCF sampling was performed. GCF is a composite mix of substances derived from serum, leukocytes, periodontal cells and oral microbes. These constituents retain a huge potential for serving as periodontal disease markers, rendering GCF a distinctive screen for investigating the periodontal status. In addition, collection of GCF is a non-invasive and a relatively simple procedure (Uitto, 2003). Furthermore, Gupta, 2013 concluded that GCF being in the immediate vicinity of periodontal tissues where periodontal disease commences leaves it more informative than saliva samples.

On the bases of the mentioned observation, the result of the present study can be explained as it detected the highest levels of chemerin in periodontitis patients with diabetes than the non-diabetic periodontitis patients followed by the healthy controls, with significant differences in chemerin level within the three groups. 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. Similarly, Patnaik et al., (2017) found that chemerin levels in GCF were greater in t2DM with periodontitis than non-diabetic periodontitis patients. These findings indicate that chemerin is produced within the crevicular fluid very close to the periodontium and therefore, may induce the chronic inflammation in patients with periodontitis. Thus, it may be blamed for playing a great role in the pathogenesis of not only DM but also periodontitis.

On the same hand, 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 accordingly, they considered chemerin as a possible marker of inflammatory activity, and essential player in the pathogenesis of the disease; this can also be attributed to the fact that chemerin acts as an important marker of an inflammatory state which is expressed by macrophages (Luangsay et al., 2009, Kaur et al., 2010). Based on the preceding findings, it is reasonable to consider that chemerin may play a central role in periodontal inflammation.

To the best of the authors' knowledge, the current study is the first to highlight the detection of FGF21 in GCF. The present investigation revealed significant greater levels of FGF21 in periodontitis patients with diabetes than the periodontitis patients without diabetes and healthy subjects. These results are aligned with Li et al., (2011); who reported significantly raised serum FGF21 concentrations in type 2 diabetes subjects, which are similar to the previous studies performed by Zhang et al., (2008), Chavez et al., (2009) and Mumtaz et al., (2015). This could be explained by the fact that describes FGF21 as a powerful metabolic regulator which plays a vital role in both glucose and lipid metabolism as suggested by Zhang et al., 2008.

Moreover, FGF21 is induced in response to varied stressful stimuli, such as inflammation, oxidative stress, hypoxia and glucose or amino acids deficiency targeting to maintain the tissue homeostasis. In addition, 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).

Similarly, Li et al., (2018) also demonstrated that FGF21 has a probable part in anti-inflammation and immunoregulation. Accordingly, treating with exogenous FGF-21 can improve LPS-induced inflammation where the mode of action of FGF-21 was attributed to the rise of IL-10. This led them to plainly assign the possible utilization of FGF21 as an interesting goal for managing inflammatory conditions.Therefore, FGF21 can be considered as an adipokine that can be utilized as a drug, drug target, or as a biomarker, depending on the physio-pathological situation (Tezze et al., 2019).

Concerning the clinical periodontal parameters, the present study detected a non-significant difference in their measurements between both of the periodontitis groups; this finding is in agreement with those of several earlier studies that detected compromised periodontal parameters among patients with type 2 diabetes (Lalla et al., 2007, Taylor et al., 2013). However, it is not in accordance with Kim et al., 2013 who related the clinical parameters to the duration of DM, which didn't investigated in the current study.

In the current study, there was no significant correlation between either chemerin and FGF21 and any of the clinical parameters (PI, GI, PD, and CAL) in both diabetic and non-diabetic patients. This disagreed with Özcan et al., 2015 who demonstrated a significant positive correlation between salivary chemerin and GI, PD and CAL supposing that chemerin plays an important role in periodontitis pathogenesis. This may be attributed to the smaller sample size included in the present study.

Regarding HbA1c level, it did not correlate with any of the clinical parameters in the present study which is in agreement with Bacic et al., 1988, who assumed no association found between the duration of diabetes and glycated hemoglobin level with the severity of periodontal disease. On the other hand, the present result is not in consistence with Lösche et al., 2000 and Agarwal et al, 2016). This may be due to the inclusion criteria adopted in the present study which included only controlled t2DM patients. Moreover, both diabetes mellitus and periodontal disease are multifactorial. Apparently, not all diabetic patients are at the same risk

for periodontal disease, and more consideration has been given to possible diabetes-related risk factors to recognize individuals who are more susceptible to periodontal disease (Heitz-Mayfield, 2005).

The present investigation found that chemerin level correlate positively with HbA1c, however, it was not statistically significant, which agreed with Halawa et al., 2018. On the other hand, there was a negative yet still not a statistically significant correlation between FGF21 and HbA1c level which was in contrast to Elhini et al., 2017. This could be attributed to the proinflammatory functions presented by chemerin and the anti-inflammatory and immune-regulatory effects presumed to be offered by FGF21 respectively. That is why further studies with larger sample size are needed for additional comprehension of the exact role of both chemerin and FGF21 on the glycemic control of diabetic patients.

In the present study, in order to discriminate between patients with healthy periodontium and those suffering from periodontitis whether systemically healthy or having diabetes, also, to differentiate between periodontitis patients whether diabetic or not, and to determine the cut-off values for both markers, the ROC curve presentation for GCF chemerin and FGF21 revealed that for both, the area under the curve (AUC) is 1 showing the highest sensitivity (100%) and specificity (100%) levels. This exceeded that of a study conducted by Özcan et al., 2015 to test the diagnostic value of salivary chemerin in periodontal disease where the AUC was 0.757 at (P<0.001). This could be explained by the fact that the close approximation of the GCF to the periodontal tissues where periodontal disease initiates, offers more information than that provided by salivary biomarkers (Gupta, 2013). As we mentioned before and up to the authors' knowledge this is the first study to shed the light on the level of FGF21 in any of the oral fluids, that's why we could not compare our results with previous studies in terms of FGF21. This analysis proposes that both of the investigated biomarkers may aid in diagnosis of periodontal disease with the highest diagnostic accuracy.

Consequently, based on the current investigations, crevicular levels of both of chemerin and FGF21 can be regarded as biomarkers for periodontal health/disease

suggesting they play a great role in the pathogenesis of periodontal disease, in addition to, their involvement in the association between periodontitis and diabetes. Nevertheless, additional large-scale studies are recommended to be performed to further verify the efficacy of these biomarkers, including uncontrolled diabetic patients with periodontitis, as the small sample size and lack of an uncontrolled diabetic group are among the limitations of this investigation.

Conclusion

GCF FGF21 and chemerin are highly sensitive and specific biomarkers which may be used as a possible non-invasive technique for periodontal disease diagnosis, suggesting a role of these adipokines in the pathogenesis of periodontal disease as well as in the connection between periodontal disease and diabetes.

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