Levels of angiopoietin 2 and high sensitivity c reactive protein in stage III periodontitis with and without type 2 diabetes mellitus – a cross sectional study

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Abstract

Aim: The aim of this study was to evaluate and correlate the levels of Angiopoietin 2 and high sensitivity-C Reactive Protein in Gingival Crevicular Fluid and serum with periodontal clinical parameters in stage III periodontitis with and without type 2 Diabetes Mellitus.

Methods: This present study of 3 months involved 44 (22 males, 22 females) age- and gender- balanced individuals divided into three groups. Group 1 consisted of 10 healthy individuals, Group 2 had 17 stage III periodontitis individuals and Group 3 consisted of 17 individuals with stage III periodontitis and type 2 DM.

Results: Group 3 was found to have significantly higher levels of Angiopoietin 2 and hs CRP in GCF and serum than the other 2 groups. A significant correlation was found between the GCF & serum levels of Angiopoietin 2 & hs CRP and periodontal clinical parameters in Groups 2 & 3.

Conclusion: Individuals with peiodontitis and type 2 DM had the highest GCF and serum levels of Ang2 & hs CRP followed by individuals with periodontitis. The present study showed that the Ang2 and hs-CRP concentration in GCF increases proportionally with the progression of periodontitis, i.e., gingival inflammation and periodontal clinical parameters and in DM.

Keywords: Chronic periodontitis; Biomarker; type 2 diabetes mellitus; Clinical study (Clinical trial); gingival crevicular fluid.

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Introduction

Angiogenesis is defined as the formation of new blood vessels from the endothelium of pre-existing vessels (Szekanecz Z *et al*. 2007). It is a complex process that involves endothelial cell (EC) division, selective degradation of vascular basement membranes and of surrounding extracellular matrix, and EC migration (Folkman J *et al*. 1992). This process has a significant role in the etiology, progression, and repair of inflamed tissues (Folkman J 1995, Folkman J *et al*. 1996). Angiogenesis is an essential process in the development of chronic inflammatory diseases (Guneri P *et al*. 2004) and contributes to the degree of the inflammation as a result of the ability of new blood vessels to transport proinflammatory cells to the lesion and supply oxygen and nutrients to the inflamed tissues (Lakey LA *et al*. 2000).

Periodontitis is a chronic inflammatory disease of richly vascularised supporting tissues of the teeth. It has active as well as quiescent period during its course. The number of blood vessels increases during the progression of periodontal disease, which has been suggested to either enhance the severity of inflammation (Johnson RB *et al*. 1999) or promote healing of the inflamed site (Cetinkaya BO *et al*. 2007). Although, in both, inflammation and healing, angiogenesis is a prominent feature, and then also there is limited information about role of angiogenesis in periodontal lesions. (Johnson RB *et al*. 1999, Prapulla DV *et al*. 2007, Chapple CC *et al*. 2000, Cornelini R *et al*. 2001, Oyama T *et al*. 2000, Yuan K *et al*. 2000, Kasprzak A *et al*. 2012, Lester SR *et al*. 2009, Yuan K *et al*. 2000)

Diabetes is a metabolic disease that, due to disturbances in insulin production, leads to abnormal fat, sugar, and protein metabolism and resultant hyperglycemia that can ultimately induce diverse multiple systems pathologies (Winer N *et al*. 2004). Periodontitis is one of the most common oral problems observed in diabetes mellitus (DM), and many studies suggest the relationship between periodontitis and DM (Katz J 2001, Kinane DF *et al*. 2001, Moore PA *et al*. 1999, Noack B *et al*. 2000, Taylor GW 2001, Oliver RC *et al*. 1994). In fact, Periodontitis has been referred to as the sixth complication of Diabetes Mellitus (DM) (Loe H 1993). Diabetes has been unequivocally confirmed as a major risk factor for periodontis (Preshaw PM 2012). There is a greater likelihood of reduction in the risk and severity of periodontitis on improving glycemic control (Preshaw PM 2012). Diabetic subjects with periodontal infection have a greater risk of worsening glycemic control over time compared to diabetic subjects without periodontitis (Taylor GW *et al*. 1996). An association between Type 2 diabetes and decreased insulin sensitivity on one hand and increased inflammation on the other has been suggested by Duzagac E *et al*. 2015 and Herder C *et al*. 2015. Type 2 diabetes and decreased insulin sensitivity lead to hyperglycemia that in turn results in the production of advanced glycation end-products in various tissues including the periodontium, which trigger inflammatory cytokine production, thus predisposing for inflammatory conditions such as periodontitis. It is therefore plausible that type 2 diabetes and decreased insulin sensitivity impact periodontal health & periodontal disease progress.

Diabetic patients have a 2-3-fold higher risk of developing severe periodontitis and progressive periodontal disease (Taylor GW 2001) and mechanisms such as vascular changes, neutrophil dysfunction, altered collagen synthesis, and genetic disposition may play a role in this increased risk (Oliver RC *et al*. 1994). Gingival crevicular fluid (GCF) is a potential pool and source of several biomarkers that could correctly indicate the severity of periodontal disease and could also reflect the status of systemic health (Silvana P. Barros *et al*. 2016).

Because of the gingival microangiopathy, oxygen supply and diffusion removal of metabolic end products, leukocyte migration and diffusion factors are impaired in diabetic patients, consequently leading to tissue repair and regeneration inability (Murrah VA 1985). Our understanding has been increased in recent years regarding the molecules associated in the pathogenesis of diabetic microvasculopathy.

Ang2, an angiogenic peptide, activates endothelial cells and increases vascular inflammation. It functions as an autocrine mediator of the endothelium and is stored predominantly in endothelial cells (Fiedler U *et al*. 2006). Ang2 is a ligand of the tyrosine kinase receptor, Tie-2, and antagonises the Ang1 induced Tie-2 receptor autophosphorylation responsible for the maintenance of endothelial cell quiescence (Yuan HT *et al*. 2009). This results in endothelial cells being sensitized to the effects of pro-inflammatory cytokines and Vascular Endothelial Growth Factor (VEGF), resulting in a loss of endothelial cell quiescence and an increase in vascular activation and inflammation. Growing evidence suggests an involvement of Ang-2 and its receptor Tie-2 in the pathophysiology of different vascular and inflammatory diseases such as arteriosclerosis (Marti HH *et al*. 1999), hypertension (Nadar SK *et al*. 2005), idiopathic pulmonary arterial hypertension (Kumpers P *et al*. 2010), chronic kidney disease (David S *et al*. 2010), and rheumatoid arthritis (DeBusk LM *et al*. 2003).

Plasma Ang2 (but not Ang1), like VEGF levels, are selectively elevated in patients with diabetes and are associated with indexes of endothelial damage/dysfunction (Lim HS *et al*. 2004, Lim HS *et al*. 2005). Ang2 plays a critical role in diabetic retinopathy as it is found

to be upregulated in diabetic retina in rats (Hammes HP *et al*. 2004) and humans (Cai J *et al*. 2008) In an immunohistochemistry study, Yuan K *et al*. 2000 reported that positive detection rate of Angiopoietin 2 were significantly higher in periodontitis and pyogenic granuloma subjects than healthy subjects.

Some inflammatory biomarkers such as cytokines, chemokines and bone-related factors have also been found to play a very important role in the pathogenesis of both periodontitis and type 2 DM (Engebretson SP *et al*. 2004, Engebretson SP *et al*. 2006). Studies of the links between periodontal disease and cardiovascular disease have indicated that C-reactive protein (CRP) is one the inflammatory biomarkers involved (Pradhan AD *et al*. 2002). CRP is an acute-phase reactant synthesized by the liver in response to the inflammatory cytokines IL-6, IL-1, and tumor necrosis factor-alpha (TNF-α). The level of circulating CRP is a marker of systemic inflammation, and is associated with periodontal disease (Noack B *et al*. 2001). Several studies conducted in the past support a significant association between Angiopoietin 2 and inflammation via CRP (Volkova E *et al*. 2011, Porta C *et al*. 2009, Turu MM *et al*. 2008). A recent review by Akwii RG *et al*. 2019 indicates that Ang2 plays a vital role in endothelial cell physiology and plays a central role in vascular related diseases.

With the increasing incidence of diabetes in an all age groups particularly in India, determination of Ang2 levels in periodontitis patients with DM and its association with inflammatory biomarker CRP may be beneficial in establishing appropriate health/oral care.

Till now, the levels of Ang2 and hs-CRP in GCF and serum in stage III periodontitis (Papapanou *et al*. 2018) with type 2 DM and periodontitis without type 2 DM have not been explored. Thus, considering the aforementioned findings the present study was designed to evaluate and correlate the levels of Ang2 and hs-CRP, in GCF and serum with periodontal clinical parameters in CP stage III periodontitis subjects with and without type 2 DM.

Materials and Methods

This cross sectional study of 3 months duration was conducted from December 2013 until February 2014 and involved 44 (22 males, 22 females) age- and gender- balanced subjects divided into three groups. The subjects were selected from among patients referred to the Department of Periodontics, Government Dental College and Research Institute, Bangalore.

Ethical clearance was approved by Institutional Ethics Committee and Review Board, Government Dental College and Research Institute, Bangalore. All the subjects, who agreed to participate, voluntarily signed a written informed consent.

Inclusion criteria

The inclusion criteria for the study subjects were an age of 25-45 years with a mean age of 40 years, presence of at least 20 natural teeth with a diagnosis of stage III periodontitis based on report of Papapanou PN *et al*. 2018 (previously referred to as "chronic periodontitis" Armitage GC 1999) as based on most commonly used periodontal clinical parameters such as probing depth (PD), clinical attachment level (CAL) (Löe H 1967) as also elucidated in a systematic review by Natto *et al*. 2018 and gingival index (GI) (Glavind L *et al*. 1967), body mass index (BMI) of 18.5-22.9 kg/m2 and a waist circumference of < 90 cm (men) or < 80 cm (women) (WHO Expert Consultation 2004), diabetic patients had well-controlled type 2 diabetes, classified according to the 2011 criteria of the American Diabetic Association (ADA) and the level of glycosylated haemoglobin (American Diabetes Association 2011). Mean PD and CAL levels were recorded as mean of all 6 sites of only those teeth without any gingival recession for all the 3 groups.

Exclusion criteria

The exclusion criteria for the study subjects were consumption of tobacco in any form, consumption of alcohol, periodontal therapy within the 6 months preceding the study, presence of any other systemic disease capable of affecting the course of periodontal disease, or those who had any course of medication affecting periodontal status.

Grouping criteria

Group 1 (healthy) comprised 10 individuals with clinically healthy periodontium, GI = 0 (absence of clinical inflammation), PD ≤ 3 mm, and CAL = 0, with no evidence of bone loss on radiographs. Group 2 (periodontitis without type 2 DM) comprised 17 individuals who had signs of clinical inflammation, GI > 1, more than 30% of sites (previously referred to as "Generalized chronic periodontitis" Armitage GC 1999) showing PD ≥ 5 mm, and CAL ≥ 3 mm, and HbA1c < 6.5% with radiographic evidence of bone loss. Group 3 (periodontitis with type 2 DM) comprised 17 individuals who had signs of clinical inflammation, GI > 1, more than 30% of sites showing PD ≥ 5 mm, and CAL ≥ 3 mm, with radiographic evidence of bone loss. Mean values of PD and CAL all six (6) sites were recorded per tooth. Only subjects with well controlled (6.5% ≤ HbA1c ≤ 7%) type 2 DM were selected based on the ADA criteria for diagnosis of diabetes (American Diabetes Association 2011).

Clinical evaluation of individuals

Group allocations and sample site selection were performed by the chief coordinator (ARP). An examiner (SPS) performed the clinical evaluation and determined the clinical parameters including PD, CAL, and GI using a University of North Carolina-15 (UNC-15) periodontal probe (Hu-friedy, Chicago, IL, USA). The same examiner (SPS) also performed the radiographic evaluations and collected the GCF samples on the next day of the clinical evaluation.

Site selection and GCF collection

GCF samples were collected from two selected test sites. In Groups 2 and 3, the sites showing the greatest CAL and signs of inflammation, along with radiographic confirmation of bone loss assessed by intraoral periapical radiographs taken by the paralleling technique, were selected for sampling. One of the two sites selected per subject was used for Ang2 and the other for hs-CRP analysis. In the healthy group, to standardize site selection and obtain an adequate fluid volume, sampling was predetermined to be from the mesio-buccal region of the maxillary right first molar, in the absence of which the left first molar was sampled. First, to avoid contamination of the paper strips, the selected site was cleaned, isolated and air-dried using sterile cotton rolls, and the supragingival plaque was removed gently using a Gracey curette (Universal Gracey curette #4R/4L, Hu-friedy, Chicago, IL, USA). A standardized protocol was followed to collect GCF from all sites in all the 3 groups. The paper strips (Periopaper, Ora Flow Inc., Amityville, NY, USA) were placed gently at the entrance of the gingival sulcus/crevice until light resistance was felt (Löe H 1965), taking care to avoid mechanical injury, and left in place for 60 seconds. The absorbed GCF volume of each strip was determined by electronic impedance (Periotron 8000, ProFlow Inc.) and GCF in an average volumetric range of 0.1 µl to 1 µl was collected in all the 3 groups. Samples that were suspected to be contaminated with blood and saliva were excluded. After collection of the gingival fluid, the two periopaper strips per site that had been used to absorb GCF from each subject were pooled and then immediately transferred to microcentrifuge tubes (premarked with the biomarker name) containing 400 µL of phosphate-buffered saline and stored frozen at -70°C for subsequent analysis. Periodontal treatment (scaling and root planing) was performed for PERIODONTITIS periodontitis subjects individuals (both groups) at the same appointment after collection of GCF by an operator.

Blood collection

Two milliliters of blood was collected using a 20-gauge needle with a 2-mL syringe from the antecubital fossa by venipuncture and immediately transferred to the laboratory. The blood sample was allowed to clot at room temperature, and after 1 h serum was separated from blood by centrifuging at 3,000 × g for 5 min. The serum was immediately transferred to a plastic vial and stored at -70°C until the time of assay.

Ang2 analysis

The samples were assayed for Ang2 using an enzymelinkedimmunosorbent assay (ELISA) kit in accordance with the manufacturer's instructions. The GCF sample tubes were first homogenized for 30 seconds and centrifuged for 5 minutes at 1,500 × g to yield an eluate. The eluate was then used as a sample for ELISA estimation of Ang2. Each sample was assayed using a commercially available ELISA kit (human Ang2, RayBiotech, Inc, USA) in accordance with the manufacturer's instructions. Color development was monitored using a microplate reader until an optimum optical density was reached, then a stop solution was added and the optical density was read at 450 nm. The total Ang2 was determined in picograms (pg), and the calculation of the concentration in each sample was performed by dividing the amount of Ang2 by the volume of the sample (pg/mL).

hs-CRP analysis

The samples for CRP were measured immunoturbimetrically. The microcentrifuge tubes containing the periopaper strips and plastic vials containing serum were transferred to the laboratory for immunoturbedimetric analysis. Serum was used undiluted. The measurement range for CRP was 0-220 mg/L.

Statistical Analysis

Analysis of variance (ANOVA) was carried out for a comparison of GCF and serum Ang2 and hs-CRP levels between the groups. Power calculations were performed before the study was initiated and these calculations warranted a minimum sample size of 30. The sample size was reached at based on a previous study (Prapulla DV *et al*. 2007). Test for the validity of normality assumption using standardized range statistics was carried out, and it was found that the assumption is valid. Based on the power of the study and the confidence interval of 95% ($p<0.05$), the individuals were divided into three study groups.

Using Pearson's correlation coefficient, the relationships between Ang2 and hs-CRP concentrations and the clinical parameters were analyzed using a software program (SPSS Inc. version 10.5, Chicago, IL, USA). Differences at $p < 0.05$ were considered statistically significant. The intra-group correlation of serum and GCF concentrations of Ang2 and hs-CRP was also performed using Pearson's correlation coffecient. The mean intra-examiner standard deviation of differences in repeated PD measurements and CAL measurements obtained using single passes of measurements with a UNC- 15 probe (correlation coefficients between duplicate measurements; $r = 0.95$).

Results

Table 1 shows the data (mean ± SD) for the study population. The mean Ang2 and hs-CRP concentrations in both serum and GCF were highest in Group 3, followed by Group 2, and were lowest in Group 1. To determine the equality of means between the three groups, ANOVA was carried out (Table 2). Significant differences in the serum and GCF levels of Ang2 and hs-CRP were found between the three groups. The Pearson correlation coefficient test was applied to evaluate the correlation and statistically significant correlation exist between the serum level of Ang2 and the serum level of hs-CRP, and also the GCF values between the both. Table 3 shows the correlation coefficients and p values. The serum and GCF levels of Ang2 and GCF levels of hs-CRP were found to be significantly correlated ($P < 0.05$) with all the clinical parameters in Group 2 and Group 3. The serum concentration of hs-CRP was significantly correlated with GI in Group 2 and PD and CAL in Group 2 and Group 3. The correlations between the GCF and serum levels of the two biomarkers and clinical parameters are presented in Table 4.

Table 1. Anagraphic data of the enrolled patients and values of probing depth, clinical attachment level, serum and GCF levels of ANG 2 and hs CRP at the sites of interest (Mean + SD).

Study Group	Group 1 (n=10)	Group 2 (n=17)	Group 3 (n=17)
Age (in years)	39.40±3.86	39.59±4.53	41.24±4.15
Sex (M/F)	4/6	10/7	8/9
GI	0	2.18±0.38	2.32±0.39
PD(mm)	1.80±0.63	6.12±0.78	7.12±1.05
CAL(mm)	0	6.82±1.07	7.88±1.27
Serum Ang2(pg/ml)	1650.00±218.02	3354.12±286.82	4622.35±342.90
GCF Ang2(pg/ml)	600.00±164.18	861.76±74.01	965.88±121.86
Serum hs-CRP(mg/l)	2.32±0.58	4.15±0.52	5.56±1.03
GCF hs-CRP(mg/l)	0.56±0.25	0.78±0.18	0.86±0.29

Table 2. Results of ANOVA comparing the mean serum and GCF concentrations of Ang2 and hs-CRP among the three groups.

Study Group	Ang2				hs-CRP			
	Serum		GCF		Serum		GCF	
	F value	p-value	F value	p-value	F value	p-value	F value	p-value
Group 1								
Group 2	316.21	<0.0001*	30.80	<0.0001*	55.87	<0.0001*	4.97	0.012*
Group3								

*Significant at p< 0.05

Table 3. Correlations of serum and GCF concentrations of Ang2 and hs-CRP in each group using Spearman's rank correlation coefficient test.

Study Group	Serum		GCF	
	Correlation coefficient	p-value	Correlation coefficient	p-value
Group1	0.989	<0.0001*	0.795	0.006*
Group2	0.911	<0.0001*	0.659	0.004*
Group3	0.697	0.002*	0.944	0.004*

*Significant at p< 0.05

Table 4. Relationship of Ang2 and hs-CRP levels to clinical parameters.

	Parameters	Group1	Group2	Group3
Ang2				
Serum	GI	-	<0.0001*	0.008*
	PD	0.006*	<0.0001*	<0.0001*
	CAL	-	0.001*	0.001*
GCF	GI	-	0.006*	0.013*
	PD	0.029*	0.044*	<0.0001*
	CAL	-	<0.0001*	0.002*
hs-CRP				
Serum	GI	-	0.001*	0.068
	PD	0.009*	<0.0001*	0.012*
	CAL	-	0.011*	0.032*
GCF	GI	-	0.002*	0.002*
	PD	0.007*	0.001*	<0.0001*
	CAL	-	<0.0001*	<0.0001*

*Significant at p< 0.05

Discussion

Periodontal diseases are a complex group of diseases characterized by inflammation and the subsequent destruction of the tooth-supporting tissues. Angiogenesis is a prominent feature of inflammation and healing and although aberrant angiogenesis is associated with lesion formation in chronic periodontitis but its role in promoting the progression or healing of periodontal lesions and the mediators that contribute to angiogenesis or therapeutic agents that control the action of the mediators have not been well described (Prapulla DV *et al*. 2007, Oyama T *et al*. 2000).

The role of DM in various periodontal diseases has been extensively investigated, and an impact of periodontal inflammation on diabetic balance has also been indicated in a study by Katz J 2001). It is reported that both severity and progression of periodontal disease has been aggravated by DM (Taylor GW 2001) and especially poor metabolic control of DM has often been associated with the severity of periodontitis (Murrah VA 1985).

The structural changes characterizing diabetic microangiopathy, which may be referred to as abnormal growth and impaired regeneration, strongly suggest a role for a number of aberrantly expressed growth factors, possibly acting in combination, in the development of these complications.

Ang2, an angiogenic peptide, activates endothelial cells and increases vascular inflammation. It functions as an autocrine mediator of the endothelium and is stored predominantly in endothelial cells.27It has been reported that Ang2 regulates vascular remodelling and endothelial responsiveness to pro-inflammatory cytokines and has a crucial role in the induction of inflammation (Fiedler U *et al*. 2006). In addition, recent in vitro and in vivo studies have demonstrated that Ang-2 acts as a chemoattractant for pro-angiogenic Tie2-expressing monocyte/macrophages (Coffelt SB *et al*. 2010, Murdoch C *et al*. 2007). Results of a very recent study suggest that Ang-2 contributes to the elevated IL-6 and IL-8 levels found in systemic sclerosis by way of monocyte activation (Carvalheiro T *et al*. 2020). There is a well established role of IL-6 in periodontal disease in diabetic patients (Ross JN *et al*. 2010).

Ang2 also plays a critical role in diabetic retinopathy (Hammes HP *et al*. 2004, Cai J *et al*. 2008) and

its plasma levels selectively elevated in patients with diabetes and are associated with indexes of endothelial damage/dysfunction (Lim HS *et al*. 2004, Lim HS *et al*. 2005).

Based on these above discussed studies, we decided to investigate the role of Ang2 in the pathogenesis of stage III periodontitis with and without type 2 DM by comparing and correlating its levels with a marker of inflammation (in this case, hs-CRP) that has been clearly proven to play a role in inflammation in various systemic diseases. The level of circulating hs-CRP is a marker of systemic inflammation, and is associated with periodontal disease (Noack B *et al*. 2001). Recent studies support a significant association between Ang2 and inflammation via CRP (Volkova E *et al*. 2011, Porta C *et al*. 2009, Turu MM *et al*. 2008).

It was anticipated that such a comparison and correlation would further validate the role of the new molecule (in this case, Ang2) being tested.

This study attempted to evaluate the GCF and serum levels of Ang2 and hs-CRP in stage III periodontitis patients with and without type 2 DM. The results clearly indicated increasing GCF and serum levels of the Ang2 and hs-CRP in patients with periodontitis and those with type 2 DM with periodontitis, relative to healthy controls.

The observed increase in hs-CRP, an acute-phase reactant protein and one of the most important markers of inflammation from Group 1 to Group 2, was in accordance with a previous study by Pradeep *et al*. 2010 in which CRP levels in GCF and serum were measured using ELISA, and also with a study by Noack *et al*. 2001.

The increase in values from health to periodontitis and further in type 2 DM with periodontitis is similar to a previous study but the levels are higher than those found in that study (Tuter G *et al*. 2007). The higher GCF and serum values in the periodontitis group could have been due to higher mean values of periodontal parameters recorded in this series of subjects. The higher mean serum values in patients with type 2 DM and periodontitis could have been due to the fact that the corresponding group in that study had coronary artery disease with PERIODONTITIS and were receiving statin therapy for the same and statins lower CRP (Tuter G *et al*. 2007). The highest serum levels of hs-CRP in group 3 could be attributed to the presence of type 2 DM in which hs-CRP levels were found to be elevated in a previous study (Noack B *et al*. 2001).

Preshaw PM *et al*. 2012 in their review have also suggested a two way inter-relationship between periodontitis and type 2 diabetes. Thy have also suggested that not only is diabetes a risk factor for periodontitis, but periodontitis could have a negative effect on glycaemic control. Thus it is possible that co-existence of stage III periodontitis and type 2 DM in group 3 in the present study could be triggering a vicious cycle of periodontal and systemic inflammation which could have reflected in highest serum and GCF levels in this group.

The increase in the GCF levels of Ang2 from Group 1 to Group 2, corresponds to a previous study by Yuan K *et al*. in which the GCF levels of Ang2 were found to be highly elevated in patients with periodontitis as compared to those with chronic gingivitis and healthy individuals (Yuan K *et al*. 2000). The GCF concentration was the lowest in periodontally healthy individuals, followed by those with periodontitis, and was highest in patients in periodontitis with type 2 DM. This could be related to the fact that Ang2 is clearly implicated in periodontal disease severity (Yuan K *et al*. 2000). The higher production of Ang2 in the serum of PERIODONTITIS patients as compared to healthy subjects could have resulted from spillover of the increased GCF Ang2 levels from diseased periodontal tissues, leading to a concomitant increase in serum Ang2. The further increase in the levels of Ang2 in serum and GCF of PERIODONTITIS patients with type 2 DM is in accordance with the studies by Lim HS *et al*. 2004, Lim HS *et al*. 2005 and Cai J *et al*. 2008. The present study showed that the GCF and serum levels of Ang2 and hs-CRP were significantly correlated with PERIODONTITIS ($p < 0.05$) and this data supports the fact that significant association exist between Ang2 and inflammation via CRP (Volkova E *et al*. 2011, Porta C *et al*. 2009, Turu MM *et al*. 2008).

Ang2 is vital for endothelial cell physiology and plays a central role in vascular-related diseases, by regulating endothelial permeability and angiogenic functions. In a review by Akwii RG *et al*. 2019, the authors summarized the current knowledge on Ang2-induced effects on blood and lymphatic endothelial cells, its role in vascular-related diseases, and provided a general overview of Ang2-induced signaling pathways in endothelial cells. They concluded that Ang2 interacts with different proteins and has diverse context-dependent effects on different cell types, which have not yet been fully elucidated.

To our knowledge this is the first study to have evaluated and correlated Ang2 and hs-CRP in stage III periodontitis with and without type 2 DM. One limitation of this trial was the small sample size evaluated. Further long-term longitudinal studies with larger sample sizes should be undertaken to validate these results. Another limitation of this study was the use of perio paper strips for GCF collection as their use could result in slight differences in the volume of GCF collected which in turn could lead to a difference in the quantity of polar molecules collected thus giving different electronic impedance on Periotron 8000. Use

of standardized microcapillary pipettes (1-5 µl) with standard premarkings is a better method to collect and record standardized GCF volumes. Based on the present findings, it can be proposed that Ang2 and hs-CRP play significant roles in the pathogenesis of periodontal disease. The highest levels of these two mediators in periodontitis with type 2 DM may indicate an active inflammatory process, both locally in periodontal tissues, and also systemically.

Conclusion

The present study showed that the Ang2 and hs-CRP concentration in GCF increases proportionally with the progression of periodontal disease, i.e., gingival inflammation and CAL and in DM.

Thus, within the limits of the present study, the role of Ang2 as a biochemical marker of periodontal disease and its progression could be proposed. However, long-term prospective studies involving a larger sample size need to be carried out to confirm the above findings. In addition, chair-side diagnostic tests and Ang2-specific therapeutic strategies could be developed to arrest the periodontitis-associated alveolar bone destruction. Also, given the active role of Ang2 in many diseases with an inflammatory response, targeting of the Ang2/Tie pathway could be a promising approach in the treatment of periodontitis and type 2 diabetes mellitus.

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