Diabetes & Metabolic Syndrome: Clinical Research & Reviews 13 (2019) 1609–1613

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original Article

Serum sTWEAK levels in chronic periodontitis and type 2 diabetes mellitus

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ARTICLE INFO

Article history:
Received 16 February 2019
Accepted 14 March 2019

Keywords:
Chronic periodontitis
Diabetes mellitus
Type 2
Cytokine TWEAK
Serum
Biomarkers

ABSTRACT

Aim: The two-way relationship between diabetes mellitus and periodontitis has been extensively studied with various interconnected biomarkers sharing a link. Soluble Tumour Necrosis Factor-like Weak inducer of apoptosis (sTWEAK) is gaining attention as an important mediator in chronic inflammatory diseases. Thus, the aim of this study was to detect, estimate and compare the levels of sTWEAK in the serum of health, chronic periodontitis (CP), and CP with type 2 diabetes mellitus (T2DM).
Materials and methods: Forty-five participants between 18 and 65 years were divided into groups of 15 each as Group 1: healthy, Group 2: CP, and Group 3: CP + T2DM. Clinical periodontal parameters and glycemic status were assessed. sTWEAK in serum was estimated using a commercially available ELISA kit. The data was statistically analyzed.
Results: sTWEAK was detected in all participants. Significant differences were observed between the groups for sTWEAK; highest in health, lower in CP and lowest in CP + T2DM. In the diseased groups, the clinical and glycemic parameters correlated positively with each other, whereas sTWEAK correlated negatively with each of the parameters.
Conclusion: The literature reports lower concentrations of systemic sTWEAK in T2DM which may be comparable to our observations in CP + T2DM when compared to health and its negative correlation with all the parameters suggesting an association with both clinical periodontal parameters and glycemic levels. However, serum sTWEAK levels may not be necessarily elevated in periodontitis as previously reported, and hence has the potential to be studied extensively for clarification with its association with T2DM.

1. Introduction

Chronic periodontitis (CP) is a complex inflammatory disease represented primarily by a poly-microbial oral infection [predominantly Gram negative anaerobic bacteria] that leads to gingival inflammation and destruction of periodontal tissues [1]. Although CP is initiated by bacteria, the host response plays an important role in the inflammatory/immunological responses in CP [2]. A mechanism exists between bacterial stimulation and tissue damage i.e., production of pro-inflammatory mediators which collectively contribute to periodontal tissue destruction [3].

Type 2 diabetes mellitus (T2DM) is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia as well as carbohydrate, fat and protein metabolism derangement because of defects in insulin secretion, action, or both. A bidirectional relationship in the pathogenesis of CP and T2DM with inflammation and the action of various molecular mediators including cytokines common to both has been reported [4,5].

Tumour necrosis factor-like weak inducer of apoptosis (TWEAK) is described as a member of the tumour necrosis factor TNF super family [6], which plays a significant role in inflammation. It is expressed by most of the cells involved in inflammation such as macrophages/monocytes, T-cells, plasma cells and also fibroblasts. It can exist in two forms, i.e., a full-length membrane-associated (mTWEAK) and a soluble (sTWEAK) type. TWEAK ligates with its receptor fibroblast growth factor-inducible protein (Fn14), bringing

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https://doi.org/10.1016/j.dsx.2019.03.027
1871-4021/

about a range of biological effects by various cell types [7,8]. The signalling mechanisms lead to the activation of several pro-inflammatory mediators such as interleukin (IL)-6, IL-8, matrix metalloproteinase (MMP)-1 and regulated on activation, normal T cell expressed and secreted (RANTES) protein. Apart from this, it also regulates immune responses and angiogenesis, tissue repair/regeneration, apoptosis, with an effect on osteoblast and osteoclasts [9].

CP and T2DM have chronic low grade inflammation as a common pathologic platform. Because altered expression of sTWEAK in these two diseases has been demonstrated, this may be an additional factor contributing to pathogenesis of CP and T2DM [10,11].

Therefore, the objective of the present investigation was to detect, estimate, analyze quantitatively and compare the serum levels of sTWEAK in health, CP and CP with T2DM, which to the best of our knowledge is a first of its kind study.

2. Materials and Methods

This cross sectional study was conducted in Department of Periodontics, S.D.M College of Dental Sciences and Hospital, Dharwad, India. An ethical clearance was obtained from the institution's ethical committee and also, a written informed consent from all the participants prior to the investigation. The study was in accordance with the World Medical Association Declaration of Helsinki.

Forty five volunteers of both the sexes, aged 18–65 years were recruited. The inclusion criteria were: subjects with at least 20 natural teeth, having CP defined as probing pocket depth (PPD) $\geq$ 5 mm, clinical loss of attachment (CAL) of $\geq$2 mm, radiographic evidence of bone loss and patients with CP + T2DM (where T2DM for this study would have been diagnosed as having random blood sugar {RBS}of $\geq$200 mg/dl and HbA1C {glycated haemoglobin} of $\geq$7.5, at least since 1 year without any diabetic complications or co-morbid conditions). The exclusion criteria were: individuals with any systemic disorders, presence of any disease that may alter the immune system, individuals who were on antibiotics and/or anti-inflammatory drug regimen, pregnant/lactating women and tobacco smokers.

Subjects were selected for each group after a thorough and precise medical and dental history, clinical examination and evaluation. A single examiner carried out the clinical measurements. All the participants were subjected to the recording of Oral Hygiene Index-Simplified (OHI–S) [12], Gingival Index (GI) [13], Plaque Index (PlI) [14], Bleeding on Probing (BOP), PPD and CAL using a UNC-15 periodontal probe[1] by a single examiner, except for estimation of RBS and HbA1C which was done by the concerned specialist in the hematology laboratory. On the basis of these clinical and laboratory parameters for CP and T2DM the participants were divided into three groups. Group 1: 15 systemically and periodontally healthy participants; Group 2: 15 CP patients who were systemically healthy; Group 3: 15 patients with CP and T2DM.

For collection of the blood sample, the skin over the antecubital fossa was disinfected and 2 ml of blood was collected by venepuncture using a 20 gauge needle with a 2 ml syringe in a test tube from all participants. The serum was then extracted from blood and stored at −80°Celsius till the assay procedure. The samples were then assayed for levels of sTWEAK by using a commercially available ELISA kit[2] as per the manufacturer's instructions. Statistical analyses of the parameters were done based on the normality of distribution using the Kolmogorov Smirnov/Shapiro-Wilk tests, followed by the one-way ANOVA, Tukey's multiple post hoc and Karl Pearson's correlation tests. The probability value was set as $p < 0.05$. The IBM-SPSS[3] software was employed for the analyses.

3. Results

Twenty-three males and 22 females with a mean age in years of 45.51 (SD ± 5.77) constituted the total of forty five participants. The demographic, clinical, haematological and sTWEAK data with the significant differences by one-way ANOVA are depicted in Table 1.Tables 2 and 3 show the pair-wise comparison of the three groups by Tukey's post hoc analysis and the overall correlation analysis by Karl Pearson's test, respectively.

OHI–S was significantly worse in Group 3 as the score was the highest when compared with Groups 1 and 2. Similar results were obtained with regard to PlI scores and GI scores**.** The PPD and CAL were higher in Groups 3 and 2 as compared with Group 1. BOP scores were also highest in Group 3, followed by Group 2 and Group 1. A pair wise comparison of BOP between Group 2 and Group 3 was not statistically significant**.**

sTWEAK values showed a lowest estimation of 107.5 ng/ml in Group 3and a highest estimation of 298.5 ng/ml in Group 1. The mean value of sTWEAK was highest in Group 1 and lowest in Group 3**.** A statistically significant difference for sTWEAK was observed between the groups when pair-wise comparisons were done. sTWEAK differed significantly between Groups 1 versus 2, and Groups 1 versus 3. No significant difference was noted between Groups 2 and 3.

With regard to the overall correlation using Karl Pearson's test, all the clinical and glycemic parameters correlated positively with each other, whereas sTWEAK correlated negatively with each parameter which was statistically significant**.** The Karl Pearson's correlation test was also applied to each group. The correlation of sTWEAK did not differ significantly with any of the parameters in Group 1**.** Significant correlations were observed in Groups 2 and 3, where HbA1C and RBS and the clinical parameters correlated positively with each other, and sTWEAK showed a negative correlation with the clinical and glycemic parameters (Supplementary Tables 1–3)**.**

4. Discussion

This study aimed to detect, estimate and compare the levels of sTWEAK in the serum of healthy participants, systemically healthy CP patients, patients with CP and T2DM, and to ascertain a plausible association with serum levels of sTWEAK.

Serum concentrations of sTWEAK were evaluated to reveal any influence of periodontal diseases as a source of systemic inflammatory burden in CP and CP with T2DM when compared to a healthy population. sTWEAK was estimated using a commercially available ELISA kit as it provides sensitive estimates. The ELISA used in this study allowed accurate quantitative estimation of sTWEAK (the limit of detection being 13.658 ng/ml, as per the manufacturer) and was detected in all the participants.

This study evaluated the OHI–S, PlI, GI, PPD, CAL, BOP, HbA1C, RBS and serum sTWEAK in each subject.

Inadequate personal oral hygiene maintenance leading to accumulation of dental plaque biofilm has been established as a major risk factor of periodontal diseases. OHI–S score was significantly worse in Group 3 as compared with Groups 1 and 2. This is in accordance with the recent systematic review and meta-analysis done by Lertpimonchai et al. [15], where a multivariate random-

[1] Hu-Friedy® Manufacturing Inc., Chicago, IL, USA.
[2] Krishgen Biosystems, Mumbai, India.
[3] IBM-SPSS, Armonk, NY, USA.

Table 1
Demographic data and Mean [±SD] values of the parameters and significant differences by One-Way ANOVA between the three groups.

Parameter	Group 1	Group 2	Group 3	*p*-value by One-way ANOVA
Sex [M/F]	8/7	7/8	8/7	0.482
Age	43.73 ± 5.02	45.86 ± 6.73	46.93 ± 5.35	0.697
OHI–S	0.84 ± 0.64	1.79 ± 0.34	3.58 ± 0.98	<0.001[a]
PlI	0.53 [±0.27]	1.65 [±0.19]	2.64 [±0.39]	<0.001[a]
GI	0.51 [±0.19]	1.57 [±0.12]	2.57 [±0.29]	<0.001[a]
BOP	0.15 ± 0.11	0.77 ± 0.13	0.84 ± 0.07	<0.001[a]
PPD	1.36 ± 0.11	6.33 ± 0.88	7.13 ± 0.86	<0.001[a]
CAL	1.36 ± 0.11	6.34 ± 0.92	7.13 ± 0.92	<0.001[a]
HbA1c	4.22 [±0.66]	5.31 [±0.14]	8.84 [±0.65]	<0.001[a]
RBS	105.85 [±11.398]	113.56 [±12.64]	236.42 [±14.07]	<0.001[a]
sTWEAK [ng/ml]	279.95 [± 15.79]	208.79 [±32.75]	191.87 [±30.40]	<0.001[a]

OHI–S: Oral Hygiene Index-Simplified, PlI: Plaque Index, GI; Gingival Index, BOP: Bleeding On Probing, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, HbA1C: Glycated haemoglobin, RBS: Random Blood Sugar, sTWEAK: soluble Tumour necrosis factor-like weak inducer of apoptosis.
[a] Statistically significant [$p < 0.05$].

Table 2
Pair-wise comparison of the three groups by Tukey's post hoc analysis.

	OHI–S	PlI	GI	BOP	PPD	CAL	HbA1c	RBS	sTWEAK
G1 vs G2	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]
G1 vs G3	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.001[a]
G2 vs G3	<0.001[a]	<0.001[a]	<0.001[a]	0.2100	<0.001[a]	0.0170[a]	<0.001[a]	<0.001[a]	0.2190

G1: Group 1, G2: Group 2, G3: Group 3, vs: versus, OHI–S: Oral Hygiene Index-Simplified, PlI: Plaque Index, GI; Gingival Index, BOP: Bleeding On Probing, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, HbA1C: Glycated haemoglobin, RBS: Random Blood Sugar, sTWEAK: soluble Tumour necrosis factor-like weak inducer of apoptosis.
[a] Statistically significant [$p < 0.05$].

Table 3
Overall correlation analysis by Karl Pearson's test.

Parameters		OHI–S	PlI	GI	PPD	CAL	BOP	HbA1C	RBS	s TWEAK
OHI–S	r-value									
	p-value									
PlI	r-value	0.81								
	p-value	[a]								
GI	r-value	0.82	0.94							
	p-value	[a]	[a]							
PPD	r-value	0.73	0.85	0.87						
	p-value	[a]	[a]	[a]						
CAL	r-value	0.73	0.84	0.87	0.99					
	p-value	[a]	[a]	[a]	[a]					
BOP	r-value	0.68	0.83	0.85	0.91	0.91				
	p-value	[a]	[a]	[a]	[a]	[a]				
HbA1C	*r-value*	0.76	0.91	0.88	0.84	0.79	0.86		0.93	
	p-value	[a]	[a]	[a]	[a]	[a]	[a]		[a]	
RBS	*r-value*	0.82	0.88	0.77	0.82	0.75	0.81	0.89		
	p-value	[a]	[a]	[a]	[a]	[a]	[a]	[a]		
s	r-value	−0.73	−0.76	−0.75	−0.85	−0.87	−0.80	−0.82	−0.79	
TWEAK	*p-value*	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	

OHI–S: Oral Hygiene Index-Simplified, PlI: Plaque Index, GI; Gingival Index, BOP: Bleeding On Probing, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, HbA1C: Glycated haemoglobin, RBS: Random Blood Sugar, sTWEAK: soluble Tumour necrosis factor-like weak inducer of apoptosis.
[a] Statistically significant [$p < 0.05$].

effects model was used to assemble the effects of fair and poor oral hygiene versus good oral hygiene on periodontitis. Their results indicated that fair and poor oral hygiene increased the risk of periodontal diseases by about two-, and five-fold, compared with good oral hygiene. Increased measures were obtained with regard to PlI scores and GI scores in the groups with diseases, which are also in agreement with the literature evaluating the effect of PlI and GI on periodontitis which have reported that higher values of PlI and GI would increase the prevalence of periodontitis [16–20].

The PPD and CAL were higher in Groups 3 and 2 as compared with Group 1. Although there were comparable estimates for both PPD and CAL in the groups 2 and 3, each of the parameters was significantly higher in the diseased groups as against health highest in Group 3 and also, BOP scores were highest in Group 3 followed by Groups 2 and 1.

sTWEAK is reported to be a novel inflammatory mediator expressed by most of the inflammatory cells [6]. sTWEAK is formed after proteolytic cleavage by a furin endoproteinase and binds to Fn14, which is its only true signal transducing receptor [7]. Fn14 has biological effects through ligation and is expressed by many cell types including epithelial cells, mesenchymal cells, endothelial cells [21–27], and osteoblasts [28]. sTWEAK and Fn14 together bring about a variety of actions like production of pro-inflammatory cytokines [29,30], and regulation of immune responses [9,31].

Regarding the association of sTWEAK in periodontal diseases,

data in the literature point to its higher expression in gingival fibroblasts and periodontal tissue [32–34]. A study by Leira et al. [35] indicates increased serum sTWEAK levels in periodontitis patients. This is contrary to our observations of low sTWEAK concentrations in CP as compared with health.

It has been observed by Blanco-Colio et al. [36], that sTWEAK levels in the plasma were decreased in patients with carotid atherosclerosis. The decreased sTWEAK levels could be because of upregulated Fn14 expression in injured vessels [37]. Vascular/endothelial damage in the systemic circulation due to periodontal infection [involving Gram negative anaerobic bacteria] has been reported [38,39]. Hence, based on the preceding information, it is our guarded hypothesis that periodontitis as a local infection affects systemic vascular injury leading to higher Fn14 secretion and a consequent low serum sTWEAK concentration. Therefore our study differs from the evidence in the literature [35], that serum sTWEAK is higher in periodontitis.

In T2DM, a dysregulation of immune responses involving cytokines brings about a heightened systemic inflammatory state through direct effects on immune cell function. Several cytokines are associated in modulating inflammation in hyperglycemia, insulin sensitivity and T2DM [40–42]. CP has the potential to alter the serum levels of cytokines which may aggravate the inflammatory status in T2DM [43,44]. Of the several pathways, one is mediated by sTWEAK [6]. Serum sTWEAK concentrations are decreased in gestational diabetes [45], type 1 diabetes [46], and T2DM [47], wherein the latter report is in similar to our study.

In our investigation, sTWEAK concentrations were negatively associated with glycemic parameters which are comparable to the study by Kralisch et al. [47], who observed lower levels of sTWEAK in T2DM. sTWEAK was shown to be a negative modulator of the signalling mechanism of TNF-α. Vazquez-Carballo et al. [48], mention that TNF-α induced insulin resistance by sTWEAK in T2DM may improve as an effect of JNK1/2 phosphorylation influenced by PP2A phosphatase which might be associated with the protective role of sTWEAK in T2DM and have proposed that in T2DM, decreased sTWEAK may sustain a pro-inflammatory effect. It has to be noted that sTWEAK has also been referred to as a dual or multifunctional cytokine [49,50].

5. Conclusion

Within the limitations of our study [such as a small sample size, C-reactive protein, Fn14, or other cytokines not estimated], serum sTWEAK was lower in CP alone and in CP + T2DM as compared with health, tempting us to consider that decreased sTWEAK may possibly support a systemic pro-inflammatory status.

To the best of our knowledge, there are no comparative studies quantifying serum concentrations of sTWEAK in health, CP and CP + T2DM. So, no direct comparisons can be made, but our results show lower serum sTWEAK in CP and CP + T2DM.

The variability of sTWEAK levels in the literature makes it challenging to attribute a definitive quality to this inflammatory mediator. It is a matter of conjecture whether CP has an additional role as a local inflammatory burden in the expression of sTWEAK in T2DM. Further research is warranted to confirm the findings of the present study to better understand the behavior of sTWEAK as a potential biomarker in the pathogenesis of periodontal diseases and type 2 diabetes mellitus.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.03.027.

Conflicts of interest & funding

The authors declare no conflicts of interest and sources of funding.

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