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Original Article

Association between GCF Sclerostin level and Haemoglobin A1c in stage III periodontitis patients with controlled and uncontrolled type 2 Diabetes Mellitus: A case-control study

Aya Ali Ahmed Hussien[1], **Ebtehal Mohammed**[2]

[1]Oral Medicine and Periodontology Department, Egyptian Russian University, Cairo, Egypt.

[2]Oral Medicine and Periodontology Department, Faculty of Dentistry, Beni-Suef University, Beni-Suef, Egypt.

Email: aya-ali@eru.edu.eg

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Abstract:

Aim: The aim of this study was to investigate the association between sclerostin level in gingival crevicular fluid and glycosylated haemoglobin in stage III periodontitis in non-diabetic, controlled and uncontrolled diabetic patients. **Methods:** 36 patients were included in this study. They were diagnosed as stage III periodontitis according to new classification of periodontal and peri-implant diseases diseases 2017. Patients were categorized into three groups with 12 patients for each group; group I (non -diabetic), group II (controlled diabetic) and group III (uncontrolled diabetic patients). Gingival crevicular fluid samples were collected from the site with greatest clinical attachment loss of each patient and sclerostin concentration was assessed by enzyme-linked immunosorbent assay. **Results**: There was no significant difference between sclerostin concentration in gingival crevicular fluid of three groups with highest concentration was in controlled diabetic patients. Also, there was no association between sclerostin concentration in gingival crevicular fluid and glycosylated haemoglobin in all study groups. **Conclusion:** Periodontitis was suggested to have more effect on sclerostin level than diabetes mellitus, which augment the evidence of sclerostin role in periodontitis destruction and recommended the anti-sclerostin antibody as a promising treatment for periodontitis.

Key words: Sclerostin, GCF, HbA1c, diabetes mellitus, periodontitis

Introduction:

Periodontitis is an irreversible inflammation of the tooth supporting structures including gingiva, periodontal ligaments, cementum and alveolar bone. ***(Könönen et al.,2019).*** Although the microbial plaque with its vigorous pathogenic bacteria is the initiating factor for periodontitis but the major destruction occurred is due to host microbial interaction. The dental biofilm only initiates gingival inflammation, while the host immune response ultimately leads to the activation of host proteinases and subsequent tissue destruction. However, the progression of disease from gingivitis to periodontitis and disease severity are related to the ecological dysbiosis changes of the microbiome. These changes occur in response to the resultant gingival inflammatory products and tissue breakdown products. Unluckily theses tissue

breakdown products are considered enriching nutrient supply for specific microbial species, which in turn activate certain immune responses. Periodontal disease has multifactorial nature that can be modified by multiple local and systemic factors, making some hosts more susceptible to disease progression than others. Diabetes mellitus and smoking are considered the most important systemic modifying factors for periodontal disease progression (***Tonetti et al., 2018).***

Diabetes mellites (D.M) is a chronic endocrine metabolic disorder characterized by state of hyperglycaemia, insulin resistance and insufficient insulin level. It is classified to type 1, type 2, gestational and other special types of diabetes with type 2 being the most prevalent one (American diabetes association, 2018). Periodontitis and D.M type have a well-established bidirectional relationship that can be mostly attributed to the inflammatory process, making diabetes mellitus a significant risk factor of periodontitis ***(Stöhr et al., 2021).***

At the tissue level, poor glycaemic control in diabetic patients increases the expression of systemic inflammatory markers in gingival crevicular fluid (GCF), especially Interleukin 1-beta (IL-1β) and tumour necrosis factor alpha (TNF-α) that are strongly associated to periodontal disease severity and destruction (Costa et al.,2023). On the other hand, the gram- negative bacteria associated with periodontitis trigger an inflammatory response, leading to systemic elevation of inflammatory markers such as C-reactive protein. This, in turn, induce release of systemic proinflammatory cytokines which worsen the insulin resistance ***(Martínez-García et al.,2021).***

At cellular level, the inflammatory process characterizing both periodontitis and D.M alter the cellular homeostasis in similar ways explaining the relation between them. They share multiple altered cellular mechanisms including modified biologic cellular membranes, dysfunction of mitochondrial, improper energy regulation, aggression recognition and insufficient elimination of cellular debris**.** These altered mechanisms have an impact on different cellular function pathways, including those responsible for bone homeostasis, particularly receptor activator of nuclear factor-κB ligand (RANKL)/RANK/osteoprotegerin (OPG), a catabolic pathway, and Wingless-related integration site (Wnt) signalling, an anabolic pathway ***(Portes et al., 2021).***

Wnt is a large family of signaling glycoproteins, they drive a number of genes that regulate cell differentiation, proliferation, migration, polarity and apoptosis for different types of cells including osteoblast-cell lineage (Ng et al., 2019). Sclerostin is glycoprotein expressed by many cells, primarily the osteocytes. By binding the lipoprotein receptor-related protein (LRP) 5/6 on osteoblasts, it competes with canonical Wnt signalling for binding to LRP 6 decreasing the osteoblastogenesis. At the same time, it can activate RANKL inducing bone resorption by promoting osteoclasts formation. This dual action of sclerostin making it an important regulator of bone metabolism and is considered as therapeutic target for bone formation by blocking its action ***(Chatzopoulos et al., 2019).***

As an inflammatory condition, periodontitis is characterised by an increase in several proinflammatory cytokines that are induced by periodontal bacteria like *Porphyromonas gingivalis* polysaccharides (Bhuyan et al.,2022). The most significant pro-inflammatory cytokines involved in periodontal destruction are IL-1, IL-6, and TNF-α, which promote sclerostin expression with subsequent enhancement of bone resorption ***(Yang et al., 2016).***

As a metabolic disorder, D.M is characterized by accumulation of advanced glycation end products (AGEs) which are responsible for accelerated periodontal tissue destruction in diabetic patients and they upregulate sclerostin expression by osteocytes.

Additionally, sclerostin expression is upregulated by proinflammatory cytokines that enhanced by poor glycaemic control in D.M leading to advanced alveolar bone loss ***(Plemmenos and Piperi,2022).***

According to new classification of periodontal diseases 2017, the periodontitis is classified into four stages based on disease severity with considering diabetes mellitus as one of two modifying factors of periodontitis. Alveolar bone loss, as a hallmark of periodontitis, is significant in both stage III and IV ***(Caton et al., 2018).***

Sclerostin and periodontitis have been the subject of extensive *in vitro* and *in vivo* study utilising both experimental animals and human subjects. Upregulation of sclerostin was detected in periodontitis patients more than healthy and in patients with peri-implantitis, also it was significantly decreased after non-surgical periodontal therapy and the experimental trials to apply antisclerostin antibody in rheumatoid arthritis and periodontitis models had promising results ***(Fangyuan et al., 2020)***. Few studies were conducted on sclerostin level in diabetic patients with periodontitis to evaluating its level in postmenopausal women (Pinho et al., 2017) and another one compared sclerostin level in healthy and diabetic patients with periodontitis ***(Prerana et al.2022).*** Up to our knowledge, no previous study investigated the level of sclerostin in controlled and uncontrolled diabetic patients with periodontitis.

The aim of current study was to assess the association of sclerostin in gingival crevicular fluid (GCF) and glycosylated haemoglobin (HbA1c) in non-diabetic, controlled and uncontrolled diabetic patients with stage III periodontitis.

Subjects and methods

In this case-control study; a total of 36 patients with stage III periodontitis were consecutively recruited from the Periodontology Clinic, Faculty of Dentistry, Egyptian Russian University. Written informed consent was obtained from every patient before inclusion. The study was reviewed and approved by the Ethical committee of the Faculty of Dentistry, Egyptian Russian University (FD-ERU-REC) with a final registration number (FD-ERU-REC- 4).

Sample size calculation:

Based on previous studies assessing sclerostin levels in GCF (Krishna et al., 2021); A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that there is no association between GCF level of sclerostin biomarker and HbA1c in study groups. By adopting an alpha (α) level of (0.05), a beta (β) of (0.2) (i.e. power=80%), and an effect size (f) of (0.637) calculated based on the results of a previous study1; the predicted sample size (n) was a total of (27) cases (i.e. 9 cases per group). Sample size calculation was performed using G*Power version 3.1.9.72 (Faul et al., 2007).

Patient Selection:

The target sample was reached through consecutive sampling for patients in the periodontics clinic, Faculty of Dentistry, Egyptian Russian University over the period from February 2023 to June 2023.

Inclusion criteria included both genders with age range from 20 to 70 years with stage III periodontitis. Diabetes type 2 was a pre-requisite for being enrolled into group II or group III patients.

Exclusion criteria included any systemic disease other than diabetes, smoking and pregnancy as they are well known risk factors for periodontal disease ***(Genco and Borgnakke, 2013).***

Periodontal examination:

Periodontal diagnosis for all cases was achieved based on the new classification system of periodontal and peri-implant diseases 2017 (Caton et al., 2018). Patients were diagnosed as having periodontitis if they had clinical attachment loss (CAL) at two or more non-adjacent teeth (Tonetti et al., 2018). For diagnosis of stage III periodontitis, detailed periodontal examination was performed to assess CAL, radiographic bone loss and number of teeth missed due to periodontal disease. The patient was diagnosed as stage III periodontitis if they had; CAL ≥ 5mm, radiographic bone loss extending to apical third of the tooth and teeth loss due to periodontal disease < 4 teeth (Tonetti, et al., 2018). Periodontal charts for all patients were recorded by a calibrated examiner using UNC-15 periodontal probe.

All patients with stage III periodontitis were screened for type 2 diabetes mellitus using glycosylated haemoglobin (HbA1c) test in a standardized laboratory. According to the level of HbA1c (Sherwani et al.,2016), the patients were categorized into three groups:

Group I: stage III periodontitis who had no diabetes (HbA1c 4-6%)

Group II stage III periodontitis who had diabetes with good control (HbA1c 6.5 – 7.5%)

Group III: stage III periodontitis who had no diabetes (HbA1c > 7.5%)

Gingival crevicular fluid Sample Collection:

Before sample collection, sites with greatest CAL were determined. Selected sites were carefully isolated with cotton rolls to minimize salivary contamination. The GCF was collected with endodontic absorbent paper points (Meta Biomed Co. Ltd., Cheongju, Korea) which inserted deeply into the sulcus tooth for 30 s. Two strips were withdrawn from each patient to make it as one sample. The paper strips were immediately sealed in Eppendorf tubes® (Eppendorf AG, Hamburg, Germany) with a phosphate-buffered saline solution (Invitrogen, Camarillo, CA, USA). Strips visually contaminated with blood or saliva were excluded. The collected samples were diluted with a dilution factor of 250µl and immediately stored in at −80°C until the day of laboratory analysis. Sclerostin levels were analyzed using an enzyme-linked immunosorbent assay using Human Sclerostin ELISA Kit (Cat. No E3068Hu).

The statistical software for the social sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA) was used to code and enter the data. The mean and standard deviation were used to summarize the data. Analysis of variance (ANOVA) with multiple comparisons Bonferroni post hoc test was used to compare the groups. Correlations between quantitative variables were done using Pearson correlation coefficient (Chan, 2003). Statistics were considered significant for P-values less than 0.05.

Results:

The mean and standard deviation of the sclerostin concentration in GCF stage III periodontitis in non-diabetic, controlled diabetic and uncontrolled diabetic patients are shown in **table 1**, with the higher concentration of sclerostin in GCF was detected in controlled diabetic patients followed by non-diabetic patients then uncontrolled diabetic patients. The comparison between the three groups showed no significant difference; *P value* was 0.986.

The mean HbA1c concentration of group I was (4.89%), in group II was (7.01%) and in group III was (9.03 %). Pearson's correlation (r) showed no significant correlation between HbA1c and sclerostin level in GCF of all groups; *P-value* was 0.862.

Discussion:

By inhibiting osteoblasts activity and promoting osteoclastic activity, which results in the loss of alveolar bone, sclerostin, a Wnt pathway antagonist, contributes to periodontitis ***(Ashifa et al., 2021).***

Table 1: GCF concentrations and comparison between the study groups

	Periodontitis		Periodontitis/ controlled D.M		Periodontitis/ uncontrolled D.M		P value
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Sclerostin concentration (ng/ml)	107.67	17.3	108.51	19.61	107.38	15.12	0.986

Table 2: Correlation between HA1c and Sclerostin level in GCF

		Sclerostin concentration (ng/ml)
HbA1c	**r**	0.030
	P value	0.862
	N	36

Also, sclerostin was hypothesized to be related to insulin resistance in diabetes patients since the Wnt pathway is essential for cell function in the pancreas with subsequent possible altered bone metabolism and microarchitecture in diabetic patients ***(Yu OH, et al, 2017; Wędrychowicz A, et al., 2019; Singh PK, et al.,2022).***

However, no previous study investigated the association between sclerostin concentration and diabetic control in periodontitis patients, so the aim of this study was to assess the association between HbA1c and sclerostin concentration in GCF of stage III periodontitis patients in non-diabetic, controlled diabetic and uncontrolled diabetic patients.

Stage III periodontitis was selected for this study as in accordance with the new classification of periodontal and peri-implant diseases and conditions 2017, it is characterized by significant bone loss that extends to the middle third of the tooth root or beyond ***(Tonetti et al., 2018).***

Gingival crevicular fluid (GCF) offers several benefits for sampling collection for detecting the changes associated with periodontal disorders, tissue destruction, and bacterial biomarkers, as well as monitoring of treatment effects. It is non-invasive, site-specific, reliable, simple, and cost effective. The site specificity can indicate the severity of disease process before extensive clinical damage ***(Fatima et al., 2021***). In this study, the sclerostin concentration was assessed in GCF of the site with greatest CAL for each participant to be reliable for study scope.

There was accumulating evidence from earlier investigations that people with periodontitis had higher levels of sclerostin than people without periodontitis. ***(Napimoga et al., 2014; Balli et al.,2015; Rezaei Esfahrood et al., 2018; Sankardas et al.,2019; Chatzopoulos et al.,2019)***. The results of this study showed no significant difference in sclerostin between stage III periodontitis patients who are non-diabetic (107.67±17.3 ng/ml), controlled diabetic (108.51±19.61ng/ml) and uncontrolled diabetic (107.38 ±15.12 ng/ml), *p-value* was 0.986, This might be explained by taking GCF samples

from the area with the highest CAL across all groups. Additionally, the same periodontitis stage and possible identical bone destruction mechanisms

These results were consistent with Ashifa et al.,2021 with no significant difference between sclerostin level in GCF of stage III-grade B and grade C, Pinho et al.,2017 with no significant difference in serum sclerostin level between non-diabetic and diabetic postmenopausal women with periodontitis. Also, Prerana G.K, et al.,2022 study results showed no significance difference in salivary level of sclerostin between healthy non-periodontitis, periodontitis and type 2 diabetes with periodontitis patients.

On the other hand, they were inconsistent with the same study by Prerana et al.,2022 that showed significant difference in sclerostin level in GCF between study groups, this can be attributed to different periodontitis diagnostic criteria, which were according to 1999 classification, as chronic periodontitis with significant difference in clinical measurements of probing depth and CAL between periodontitis and periodontitis with DM type 2 patients, so the significant difference of sclerostin level might be attributed to disease severity of periodontitis rather than effect of D.M, while in this study all study groups had similar periodontitis severity (stage III). Also, they were inconsistent with Sakamoto et al., 2019 *in vitro* study that showed a 30 folds increase in sclerostin expression by cultured osteoblast induced by co-culture of AGEs-2 with *Porphyromonas gingivalis* lipopolysaccharide due to upregulation of toll like receptors 2, IL-6 and other proinflammatory cytokines, however in this study we assessed HbA1c not AGEs.

The results of this study showed no association between HbA1c and sclerostin level in GCF of all study groups that in consistence with Pinho et al., 2017 study that showed no significant association between serum sclerostin and HbA1c of non-diabetic and diabetic menopausal women with periodontitis, also Wędrychowicz et al.,2019's study showed no association between serum sclerostin level and HbA1c in children with type I diabetes. Although there was a significant correlation between diabetes and changes in bone metabolism and microarchitecture, there was insufficient evidence to conclusively link these changes to elevated sclerostin expression**.** ***(Pinho et al.,2017)***

On the other hand, serum sclerostin and HbA1c revealed a positive association in Singh et al.,2022. Although the source and influencing variables of serum sclerostin are diverse, the GCF in this investigation had the benefit of being site-specific, and to our knowledge, no prior study has looked into the relationship between sclerostin level in GCF and HbA1c in patients with periodontitis.

In this study results the sclerostin level in controlled diabetic patients was slightly higher than uncontrolled diabetic patients with stage III periodontitis, and this could be explained that AGEs accumulation, which have the undesirable effect on tissues, are not necessarily correlated with HbA1c which reflects recent condition of diabetic control over only three months ***(Basman et al.,2020)***

We concluded from this study that the influence of periodontitis' pathologic process is incomparable to the impact of diabetes mellitus, which has a smaller effect on the local level of sclerostin in the periodontium.

The clinical relevance of this study was to strengthen the evidence of sclerostin involvement in periodontitis and to offer a possible therapeutic alternative by preventing its activity.

One of the study's shortcomings was the small sample size and lack of analysis of sclerostin levels in the serum.

The authors recommend further research into the relationship between diabetes and sclerostin in periodontitis by evaluating the

correlation between sclerostin and AGEs in periodontitis patients with diabetes mellitus.

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The authors declare no competing interests.

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