

Article

Assessment of the Salivary level of Sphingosine kinases-1 in periodontitis and its correlation with periodontal parameters

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Available from: <http://dx.doi.org/10.21931/RB/CSS/2023.08.03.65>

Abstract

One of the key molecules in the conversion of sphingosine to sphingosine-1-phosphate is SPHK-1, also known as Sphingosine Kinase 1 (SPHK-1). Sphingosine-1-phosphate (S1P) is a lipid that acts as a signaling molecule and plays an essential role in inflammatory and immunomodulatory responses. S1P has recently been identified as a mediator and a biomarker in inflammatory bone diseases such as osteoporosis and inflammatory osteolysis based on the biological effects of S1P in osteoclastic and osteoblastic cells and immune cells. According to recent research, S1P may play a role in the pathogenesis of periodontitis, an inflammatory bone-destructive condition. This study assesses the salivary level SPHK-1 in periodontitis and its correlation with periodontal parameters. The study sample consisted of 65 participants, both males and females. It was divided into three groups: the first group, the Healthy Control group (15 Subjects). The second group, Periodontitis Stage II (25 Subjects), and the third group, Periodontitis Stage III (25 Subjects). Collection of whole unstimulated salivary samples from all participants was carried out, followed by an examination of clinical periodontal parameters (plaque index, probing pocket depth, bleeding on probing, and clinical attachment level). Then, radiographs confirmed the staging of periodontitis. Collected saliva was subjected to biomarker analysis using an enzyme-linked immunosorbent assay (ELISA) to detect the SPHK-1 level. This study found an increase in the mean SPHK-1 level with increased severity of periodontitis with a significant difference. In addition, positive weak correlations were found between the salivary SPHK-1 and the clinical periodontal parameters (PLI, BOP, PPD, CAL). The study demonstrated that the salivary SPHK-1 level can be helpful to monitor periodontal disease progression.

Keywords: Periodontitis, Saliva, Sphingosine -1 phosphate, Sphingosine kinase-1.

Introduction

Periodontitis is one of the most common infectious diseases in humans. It is characterized by inflammation of the periodontal tissue followed by the destruction of the alveolar bone, resulting in tooth loss¹. It is a significant public health problem because of its high prevalence and because it can lead to tooth loss and disability. It impairs chewing function and aesthetics, causes social inequality, and significantly impacts the quality of life. Periodontitis is responsible for a large percentage of edentulism, and masticatory dysfunction hurts overall health and costs money in dental care². Periodontitis begins and

progresses due to an interaction between pathogenic bacteria in the subgingival dental biofilm around teeth and the host response³. Bacteria can initiate immune and inflammatory responses, organizing specific cell subsets and molecules that are important in the progression of the disease⁴. Various lipid classes produced by metabolisms are relatively healthy intermediaries of inflammation and periodontal tissue destruction⁵.

Lipids with a sphingosine backbone are known as lysophospholipids and are necessary for cellular structure, communication, and metabolic activities such as cellular growth, inflammation, and healing⁶. Sphingosine kinases (SPHK1 and SPHK2) convert sphingosine into Sphingosine 1 _phosphate intracellularly⁷. SPHK-1 is a critical kinase that helps ceramide and sphingosine combine to make sphingosine-1-phosphate, a key sphingolipid signaling mediator⁸. One of the several bioactive sphingolipid products of phosphorylation of sphingosine kinase, Sphingosine-1-phosphate, is involved in a wide range of physiological functions.⁹

Sphingosine 1 _phosphate has been identified in bone remodeling processes, inducing the formation of osteoblasts and osteoclasts¹⁰. Studies have demonstrated the participation of Sphingosine 1 _phosphate in inflammatory diseases such as rheumatoid arthritis and bone homeostasis¹¹ and its induced chemotaxis and regulation of the migration of osteoclast precursors in bone tissue homeostasis¹². However, the role it plays in the osteoclastogenesis of periodontitis remains unclear.

Saliva is considered a potential reservoir of biological markers ranging from changes in biochemicals, DNA, RNA, and proteins to microbiota structure. Collecting saliva is relatively safe, reduces the risk of virus transmission, and is a simple way to aid in disease diagnosis¹³.

Because clinical studies assessing Sphingosine kinase in individual periodontium in health and disease are insufficient, throughout this study, we aimed to assess the salivary level of Sphingosine kinases-1 in periodontitis and its correlation with periodontal parameters.

Materials And Methods

This study was an observational case-control study with a human sample of 65 male and female subjects ranging in age from (30-60) years who fulfilled the study's criteria. Sample collection was done at the University of Baghdad/College of Dentistry/Department of Periodontics. Each participant was provided detailed information about the study and the procedures involved, and their informed consent was obtained on a form approved by the ethical committee of the University of Baghdad's College of Dentistry.

The Inclusion criteria included Patients with 20 teeth minimum, systemically healthy patients, patients who had not taken antibiotics and anti-inflammatory drugs in the last three months, and those aged between 30 and 60. Exclusion criteria included patients who have undergone or are currently under extensive periodontal treatment, a course of anti-inflammatory or antimicrobial therapy during the last 3 months, smoking or alcohol drinking, patients with chronic systemic disease, immunocompromised patients, pregnant, on contraceptive pills, and lactating women, patients with diseases of the soft and hard palate and mucosa such as white and red lesions and ulcers, patients wearing orthodontic appliances, removable dentures, implant, crown and bridge, patients with active Covid-19 virus infection.

The study's subjects were categorized into 3 groups: the control group with healthy intact periodontium (n=15)¹⁴, and the periodontitis patients (cases) divided into 2 groups, including stage II and III periodontitis patients (n=50)¹⁵. The control group and the periodontitis patients were as follows:

Control healthy intact periodontium (15 subjects): no probing attachment loss, Probing pocket depths ≤ 3 Mm, Bleeding on probing $< 10\%$, no Radiological bone loss¹⁴.

Stage II periodontitis (25 patients): (bone loss involving Coronal 1/3 of the root).

Stage III periodontitis (25 patients): (bone loss involving the middle 1/3 of the root).

The clinical periodontal parameters examinations were performed for all subjects by the same examiner. Periodontal parameters (PLI, BOP, PPD, and CAL) were measured by a periodontal probing instrument (the University of Michigan O probe with Williams marking at 1,2,3,5,7,8,9 and 10 mm. Six surfaces per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual), except for plaque scores where four surfaces were examined (mesial, buccal, distal, lingual). Wisdom teeth were excluded from the examination.

Saliva samples were collected from all patients and healthy control groups to evaluate the salivary level of SPHK-1. All participants were instructed not to eat or drink (except having water) at least 1-2 hours before the donation of saliva, the subject should sit in a relaxed position, and samples containing blood should be discarded. Saliva was collected between 9-12 am. After the subject rinsed his mouth several times with water to remove any debris or contaminating material before saliva collection and then waited 1-2 minutes for water clearance, 5ml of whole unstimulated saliva was collected into tubes. Three ml of saliva was centrifuged at 2500 rpm for 20 minutes, and the resulting supernatant was stored at -20°C in Eppendorf tubes until assayed.

Biomarker analysis of salivary SPHK-1 was done using a kit manufactured by SUNLONG Biotech Co., Ltd, China, using the ELISA technique.

The Statistical Package for Social Science (SPSS version 21) (Chicago, USA, Illinois) was used to describe, analyze, and present the data. Mean and standard deviation (SD) for nominal variables were calculated, as well as Inferential Statistics such as Pearson correlation (r), Levene test, two independent sample T-test, Shapiro Wilk and D'agostino Pearson test for the testing normality of quantitative variables, and One Way Analysis of Variance (ANOVA) with Games-Howell posthoc test and Tukey Kramer HSD.

Results

The current study found that the mean values of clinical periodontal parameters (PLI, BOP, PPD, and CAL) increased significantly with periodontitis severity, with the lowest values found in the control healthy periodontium group, as shown in Table 1.

The descriptive statistics for SPHK-1 analysis among study groups show that the lowest mean value of SPHK-1 was found in the control healthy periodontal group, and it increased with stages of periodontitis progression with a significant difference among groups, as shown in Table 2.

Using Pearson's correlation coefficient, it was observed that there was a weak but significant positive association between the salivary SPHK-1 and the clinical periodontal parameter in the study groups (PLI, BOP, PPD, CAL), as indicated in Table 3.

Groups	PLI	BOP	PPD	CAL
	Mean \pm S.D.			
	10.523 \pm 4.741	3.749 \pm 2.918	-	-
	78.810 \pm 8.545	40.847 \pm 12.970	4.642 \pm 0.543	3.207 \pm 0.629
	79.792 \pm 8.848	48.006 \pm 15.152	4.592 \pm 0.456	4.202 \pm 0.470
	0.000 Sig.	0.000 Sig.	0.000 Sig.	0.000 Sig.

Table 1: Descriptive and statistical test of clinical periodontal parameters among groups using Way Analysis Of Variance (ANOVA).

Groups	Mean	\pm SD	\pm SE	Mini- mum	Maximum	F	P val- ue
Control	37.338 (pg \ ML)	12.136	3.133	15.447	58.460	5.005	0.009 Sig.
Stage-II	85.755 (pg \ ML)	87.468	15.969	25.744	471.626		
Stage-III	108.682(pg \ ML)	70.037	12.787	26.496	310.667		

Table 2: Descriptive and statistical test of SPHK-1(pg/ML) among groups using One Way Analysis Of Variance (ANOVA).

Periodontal parameters	SPHK-1	
	R	P*
%PLI	0.318	0.005
%BOP	0.270	0.019
PPD	0.314	0.006
CAL	0.343	0.003

Table 3. Pearson correlation between SPHK-1 and clinical periodontal parameters

*=significant at $p < 0.05$.

Discussion

The groups in this study are periodontitis and control groups. The control group is systemically healthy without a history of any systemic disease and with healthy periodontium. So, the periodontitis group's average PLI values were significantly more significant than the other groups. These findings are consistent with those

of other studies, such as the one performed by Khamees et al.¹⁶. This is clear since plaque accumulation is the primary causative factor of periodontitis, and periodontitis is a chronic inflammation of the gingiva and connective tissue¹⁷. Furthermore, the control group was without periodontal pockets or clinical attachment loss, so no objective comparisons can be made in PPD or CAL between the study and control groups. These were due to increased bacterial invasion and the amount of plaque that resulted in the sulcular and junctional epithelium destruction and damage of surrounding alveolar bone in periodontitis¹⁸.

The biomarker analysis of SPHK-1 results revealed that the lowest mean value of SPHK-1 was found in the control healthy periodontal group, and it increased with stages of periodontitis progression, with a significant difference among groups. These results agree with Moritz, E. et al.,¹⁹ who found higher expression levels of the SphK1-generating enzyme of S1P in CD68-positive macrophages and associated immune cells in inflamed human gingival tissue compared to normal human gingival tissue, which may lead to locally elevated S1P concentrations¹⁹.

According to the Yu et al. study, periodontitis is a bacteria-driven inflammatory bone loss disease. Oral pathogens, such as *A. actinomycetemcomitans*, the pathogen associated with localized aggressive periodontitis, initiate a pro-inflammatory response leading to periodontal soft tissue damage, alveolar bone resorption, and subsequent tooth loss. This study demonstrates that the oral pathogen *A. actinomycetemcomitans* activated SPHK-1, leading to S1P generation. In vitro, deficiency of SPHK-1 significantly attenuated S1P generation induced by *A. actinomycetemcomitans* and significantly decreased the chemotaxis of monocytes and macrophages. Additionally, this study supports that SK1 and S1P play an essential role in regulating the inflammatory bone loss response induced by the oral pathogen *A. actinomycetemcomitans*¹¹.

The study found that SPHK-1 protein levels in the GCF changed significantly from disease to health, indicating that it could perform a significant role in the inflamed periodontal tissues, where SPHK1 levels were more significant in the diseased condition and gradually decreased after treatment was completed²⁰. SPHK-1 transformation of S1P has been linked to Receptor Activator Nuclear Factor KB ligand stimulation and regulating bone loss in periodontitis in rats, suggesting that SPHK-1 plays a vital role in periodontitis²¹.

Conclusion

Salivary SPHK-1 levels are significantly higher in periodontitis groups than in healthy groups, and their level increases with the severity of periodontitis, suggesting SPHK-1 can be used to monitor periodontal disease progression.

Funding:

This research received no external funding.

Conflicts of Interest:

The authors declare no conflict of interest.

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Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023

Citation: Monsor, A.A.; Akram, H.M.. Assessment of the Salivary level of Sphingosine kinases-1 in periodontitis and its correlation with periodontal parameters. *Revis Bionatura* 2023;8 (3) 65. <http://dx.doi.org/10.21931/RB/CSS/2023.08.03.65>