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Evaluation of salivary alpha amylase activity in smokers with periodontitis, Khartoum state, 2023

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ABSTRACT

Introduction and aim. Smoking widely affect oral health, including its role in the development of periodontitis. Saliva contains an antioxidant system and various enzymes. The study was designed to evaluate the activity of salivary alpha amylase among individuals who smoke and have periodontitis.

Material and methods. A total of 100 participants were included, with 50 cases (cigarette smokers with periodontitis) and the remaining 50 nonsmokers with healthy periodontium as the control group. Saliva samples were collected to measure salivary alpha amylase activity.

Results. Smokers with periodontitis had significantly higher levels of salivary alpha amylase compared to the control group (177.96±14.5 vs 94.04±19.6 IU/mL, p<0.001). Additionally, there was a weak negative correlation between the level of alpha amylase and the age of the patients (p=0.01, r=0.376). However, no correlation was found between the level of alpha amylase and the duration of smoking (p=0.584, r=0.079).

Conclusion. There is a significant increase in salivary alpha amylase levels among smokers with periodontitis. No correlation was found between age and salivary alpha amylase levels. However, a weak positive correlation was observed between the duration of smoking and salivary alpha amylase activity.

Keywords. alpha-amylase, periodontitis, saliva, smokers

Introduction

Periodontitis is an inflammatory condition that causes damage to the periodontal tissue and ultimately results in tooth loss. It can affect people of all ages but is more prevalent in adults.[1] Lifestyle factors, such as smoking and oral hygiene habits, can influence the severity and progression of chronic periodontitis, in addition to bacterial plaques.[2,3]

Smoking poses a significant risk to oral health, playing a major role in the development of cancerous and precancerous lesions, as well as periodontal disease. It is considered an independent risk factor for these canc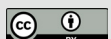ers due to the presence of toxic compounds in cigarettes, including aldehydes, carbon monoxide, hydrogen cyanide, benzopyrene, and oxygen radicals.[4,5]

Saliva serves as a protective barrier for the mouth. It consists of a complex system primarily composed of water, but it also contains low-molecular-weight enzymes, hormones, antibodies, antimicrobial ingredients, and growth factors. Some of these components are produced locally by the salivary gland, while others are transported from the bloodstream through diffusion processes, such as active transport and ultrafiltration. Saliva provides insight into overall bodily function.[6] Saliva contains various enzymes, such as lipase, peptidase, and hydrolase. Notably, the most abundant protein in hu-

man saliva is alpha-amylase, which functions as a digestive enzyme.[7]

Alpha-amylase plays a crucial role in the breakdown of starch into dextrin and malt oligosaccharides that contain α-D-(1, 4) linkages. It also facilitates the breakdown of isomaltooligosaccharides with alpha-D-(1,6) linkages, as well as the trisaccharide maltotriose and the disaccharide maltose.[8]

The addictive nature of nicotine in tobacco and the role of smoking as a preventable risk factor for periodontitis have been well-established. Hence, the measurement of salivary biomarkers can be a valuable tool for identifying individuals who may be at risk of developing or worsening periodontal disease.[9] Nevertheless, the silent nature of periodontitis poses a challenge for identifying individuals at risk until the disease has already progressed to a severe stage. Saliva offers several advantages over blood as a diagnostic fluid, including its non-invasive nature, ease of collection and storage without specialized equipment, and suitability for individuals who have difficulty with blood collection. Therefore, the current study was conducted to evaluate the activity of salivary alpha-amylase in smokers with periodontitis.[10]

Material and methods

This prospective analytical cross-sectional study was conducted at the Khartoum Dental Teaching Hospital in Khartoum, Sudan, from January to April 2023.

The aim of this study was to compare the activity of salivary alpha-amylase enzymes between smokers with periodontitis and nonsmokers without periodontitis. This research consisted of both clinical and laboratory investigations. A total of 100 participants who met the inclusion criteria and expressed interest in participating were selected from the pool of individuals.

Fifty individuals were smokers and had periodontitis (case group), whereas the control group consisted of 50 participants who had a healthy periodontium and were non-smokers. Individuals with concurrent chronic conditions such as hypertension or diabetes mellitus, as well as those taking medications, currently undergoing antimicrobial therapy, or who had received periodontal treatment within the past six months, were excluded from the study.

The study was approved by Ethical Committee of University of Medical Sciences and Technology (No. UMST/EG/2023/18), and before the clinical examination, participants were provided with a verbal explanation of the study's objectives and procedures. They were then requested to sign a formal consent form.

Periodontitis was defined as having a probing pocket depth of ≥4 mm and a clinical attachment level of ≥2 mm. The stage of the disease was not taken into consideration.

The participants were given specific instructions before collecting unstimulated whole saliva for analysis. They were instructed to wait for a minimum of 30 minutes after eating, drinking, smoking, or chewing gum. After this waiting period, they were required to rinse their mouth with water several times and wait for 1-2 minutes until the water was clear before proceeding with saliva collection. After collection, the samples were centrifuged at 4000 rpm for 10 minutes and then frozen at -20°C until α-amylase analysis was conducted. Salivary α-amylase analysis was performed using α-amylase liquicolor, a colorimetric test, from Demeditec Diagnostics GmbH in Kiel, Germany, following the manufacturer's instructions.

The data was entered and organized in a Microsoft Office Excel 2010 spreadsheet. The Statistical Package for the Social Sciences software (version 22.0; IBM SPSS Inc.) was used for analysis. The information collected from the questionnaire was coded as variables. The normality of the data was tested using the Kolmogorov-Smirnov test. Descriptive and inferential statistics, including analysis of independent variables, were then conducted.

Results

A total of 100 participants were included in the study, with a mean age of (36±7.9) years for the case group and (35±8) years for the control group. This information is presented in Table 1. The mean salivary alpha amylase was significantly higher in the cases group (177.962±14.5 IU/mL) compared to the control group (94.042±19.6 IU/mL), with a p<0.001.

Table 1. Distribution of the study group according to age (case vs. control), n=100

Age	Mean ± SD	Minimum	Maximum	p
Case (n=50)	36 ± 7.9	22	52	0.207
Control (n=50)	35 ± 8.0	19	49	

Table 2. Mean difference of amylase levels among case and control group, independent t-test, n=100

Study population	α-amylase (IU/ml)		p
	Mean	SD	
Case group (n=50)	177.96	14.5	<0.001
Control group (n=50)	94.04	19.6	

Table 3. Correlations between α-amylase activities and age, Pearson's correlation, n=50

Correlation		Age	Duration
α-amylase	n	50	50
	Correlation coefficient (r)	0.376	0.079
	p	0.01	0.584
	Strength	Weak	Weak
	Direction	Positive	Positive

There was a weak negative correlation between the salivary alpha amylase level and the age of the participants (r=-0.376). However, this correlation was found to be statistically insignificant (p=0.01, Table 2).

There was a weak positive correlation between salivary alpha amylase activity and duration of use (r=0.79). However, the correlation was not statistically significant (p=0.584, Table 3).

Discussion

Extensive research has been conducted on the oral health of smokers, particularly in relation to periodontal disease. Salivary proteins have been found to play a crucial role in the body's natural defenses against these diseases.[11]

Traditionally, periodontitis has been diagnosed through clinical examination and radiographic assessment, which can lead to measurement errors. However, changes in specific markers in oral fluids serve as valuable diagnostic tools for assessing severity and identifying individuals who are susceptible.[12] The concept that saliva reflects the overall health of the body remains valid. In recent years, researchers have utilized saliva analysis to monitor the onset, response to treatment, and outcomes of various diseases.[13] Therefore, in this study, salivary alpha amylase levels are compared between smokers with periodontitis and non-smokers with healthy periodontium.

When compared to the control group, the current study found a significant increase in salivary amylase levels in the case group. This result was consistent with the research conducted by Parlak et al. and Rashid et al., which demonstrated that periodontitis can lead to an elevated production of salivary proteins, such as mucin and amylase.[14,15] Patients with moderate to severe periodontitis had higher concentrations of these two forms of proteins. According to a study by Papacosta, salivary alpha-amylase serves as the initial defense mechanism, which aligns with the observed increase in alpha-amylase levels in periodontitis. Rohleder claims that this enzyme protects against pathogens entering the body through the mucosal surface. It may serve as the most significant marker of mucosal immunity in the oral cavity by inhibiting the attachment of bacteria.[16,17]

In contrast, some of the findings of this study contradicted those reported previously by Sequeira et al. and found no evidence that smoking was a risk factor for periodontal disease.[18] This could be attributed to the smaller sample size of 24 patients and the diverse study locations, as well as the limited availability of information regarding the severity of periodontitis and inaccurate assumptions.

Furthermore, the study revealed a weak positive correlation between the level of alpha amylase and the age of patients. These findings are consistent with a study conducted by Parlak et al., which reported a significant correlation between the age of participants and the activity of salivary enzymes.[14]

Additionally, the study showed that there was no significant correlation between alpha amylase levels and the duration of smoking. These findings are inconsistent with the previous studies. The study has limitations, such as a short duration and the absence of a periodontitis stage. We suggest developing programs to educate the community about the impact of smoking on oral health. Additionally, it is important to manipulate salivary biomarkers for the early detection and monitoring of chronic periodontitis. Furthermore, it is recommended to conduct additional studies that assess a broader range of salivary biomarkers.

Conclusion

In conclusion, the study revealed a significant increase in salivary alpha amylase activities in individuals who smoke and have periodontitis. Duration of smoking was not found to be linked to salivary alpha amylase activity. However, a weak positive correlation was observed between age and salivary alpha amylase levels.

Declarations

Author contributions
Conceptualization, H.A. and F.A.; Methodology, F.A.; Software, H.A.; Validation, H.A., and F.A.; Formal Analysis, H.A.; Investigation, H.A.; Resources, F.A.; Data Curation, H.A.; Writing – Original Draft Preparation, F.A.; Writing – Review & Editing, H.A.; Visualization, H.A.; Supervision, H.A.; Project Administration, H.A.; Funding Acquisition, F.A.

Conflicts of interest
The authors have no conflicts of interest to declare.

Data availability
All datasets are available upon request from the corresponding author.

Ethics approval
The study was approved by the ethical committees of the University of Medical Sciences and Technology No. UMST/EG/2023/18. Informed consents were obtained from all participants, and approval was granted by the hospital administration through the office of the medical director.

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