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Research Article

Comparative Analysis of Salivary Alpha-Amylase Enzyme Concentrations in Patients with Chronic Periodontitis and Rheumatoid Arthritis

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Abstract

Aims: The present study aimed to evaluate and contrast the concentrations of salivary alpha-amylase enzyme and the condition of periodontal health by the measurement of many clinical periodontal indicators, including plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL). The assessment will be carried out on several research cohorts, with an additional focus on investigating the association between alpha-amylase salivary enzyme levels and clinical periodontal parameters.

Materials and Methods: A total of 150 participants, ranging in age from 25 to 65, were observed, and this cross-sectional research was conducted. The individuals were categorized into three distinct groups according to their periodontal health status: 50 individuals with periodontitis (stage II and III, grade B), 50 rheumatoid arthritis patients (male and female) with periodontitis, and 50 Individuals who have a periodontium that is in a state of clinical health and good overall medical health. Salivary alpha-amylase enzyme levels, GI, BOP, PPD, and CAL were recorded for all attendees.

Results: The findings demonstrated substantial disparities between the research and control groups regarding clinical periodontal measures, including CAL, BOP score 1, GI, PLI, and PPD. The group with the most significant average alpha-amylase levels is Rheumatoid arthritis with periodontitis (stage II and III) (145.13). In contrast, the control group has the lowest average amylase value (77.32). The link between alpha-amylase and clinical periodontal parameters was virtually significant and positively correlated in three groups.

Conclusion: Evaluating the amount of alpha-amylase in the saliva is believed to be an excellent biological indication for predicting the development of periodontal disease in individuals afflicted with rheumatoid arthritis. Therefore, this provides a solid notion of the influence that systemic illness has on the periodontal tissues.

Keywords: Alpha-amylase, Periodontitis, Rheumatoid arthritis (RA).

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INTRODUCTION

Periodontitis is a non-reversible infection of the tissues responsible for supporting the teeth and is a widespread inflammatory illness in humans. Tooth loss is prevalent in severe manifestations of the condition and has been shown to affect over 20% of the global population (1). Chronic periodontitis is often believed to begin when a group of harmful bacteria, including *Porphyromonas gingivalis, Tamberelli forsythia, and Treponema denticola*, colonize tooth plaque. In susceptible people, the interplay of the interaction among bacterial products, cell populations, and mediators leads to the infiltration of white blood cells and the eventual deterioration of the tissues that support the tooth (2). This process is triggered by resident cells and preformed mediators. This condition is also impacted by genetic and environmental variables that increase the likelihood of specific outcomes and is classified as a complex illness with several causes. Environmental variables, such as smoking, poor dental hygiene, bacterial plaque, and stress, significantly impact the expression. Polymorphonuclear neutrophils (PMNs) are the first defensive mechanism in safeguarding the host from periodontal infections in the gingival sulcus and junctional epithelium (3,4). The data on the involvement of pathogenesis in periodontitis are inconclusive. PMNs play a crucial role in protecting against periodontitis. However, bacteria may avoid being killed by the neutrophils, and the neutrophils may take longer to die, which can change their role from defender to attacker; these cells can secrete several substances, including reactive oxygen species, collagenases, and other proteases, such as the activation caused by a diverse array of cytokines. Macrophages may function as antigen-presenting cells in this situation, facilitating the activation of lymphocytes. Consequently, the number of neutrophils in the inflammatory infiltrates diminishes when the shift from gingivitis to periodontitis occurs, with a higher presence of lymphocytes (5).

Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects the whole body and leads to inflammation and synovial membrane growth in joints with two articulating surfaces. Rheumatoid arthritis (RA), when not adequately managed,

causes significant damage to the joints, leading to impaired function, poor quality of life, and shorter lifespan, mainly owing to an increased likelihood of experiencing cardiovascular events. Rheumatoid arthritis (RA) is a complicated illness with several causes, including genetic predisposition, environmental factors, and hormonal influences (6). However, the exact cause of RA is still unclear. The present knowledge of rheumatoid arthritis (RA) etiology suggests that the principal occurrence during the first phases of rheumatoid arthritis (RA) involves the enzymatic activity of peptidyl arginine deiminase (PAD), which facilitates the conversion of arginine to citrulline. This enzymatic transformation leads to posttranslational modifications in structural proteins. While protein citrullination may occur in conditions other than rheumatoid arthritis (RA), producing anti-citrullinated protein antibodies (ACPA) is almost exclusive to this illness. These antibodies serve as a distinctive indicator of severe RA (7).

Saliva, a readily available and noninvasive substitute, has significant promise for clinical diagnostics. Salivary biomarkers, which may be generated by both healthy persons and those afflicted by certain illnesses, have become valuable tools for monitoring health, detecting the development of diseases, and evaluating their severity (Dabra et al., 2012; "; Korte & Kinney, 2016). Three Saliva is a distinctive and significant bodily fluid that may be conveniently and quickly collected without needing specialist equipment or processes. The salivary sample is a straightforward method characterized by its simplicity, non-invasiveness, and safety, and its storage is easy and cost-effective. The parotid and submandibular glands release amylase, an essential component of saliva, in response to β-adrenergic stimulation. β-Amylase functions as a significant lipopolysaccharide-binding protein in *Aggregatibacter actinomycetemcomitans* (Aa) and *Porphyromonas gingivalis* (*P. gingivalis*), and it also helps to inhibit the adhesion of *streptococcal* bacteria. (11).

Many research projects have been conducted to investigate the salivary concentrations of amylase and the connections between these concentrations and clinical parameters in patients suffering from gingivitis, periodontitis, and RA (12). In

a study conducted by Dyah Nindita et al. (2017), it was shown that the levels of alpha-amylase in the periodontitis group were elevated compared to those in the gingivitis group (13). Also, Madiha Anwar et al. (2022) revealed that the incidence of amylase increases in patients with moderate to severe periodontitis (14). Hyoun-Ah Kim et al. (2016) found evidence that suggests the concentration of alpha-amylase in patients with rheumatoid arthritis (RA) is greater than that of healthy controls, and this finding may be employed as a co-diagnostic factor (15).

This research aimed to observe the possible correlation between rheumatoid arthritis and periodontal disease. The study aims to determine the presence of a correlation between the concentration of salivary alpha-amylase and the condition of periodontal health. This is accomplished by assessing the clinical periodontal factors and comparing the salivary amylase levels to these parameters. The research aims to contribute to the current knowledge by investigating a potential correlation between two often-seen medical disorders and establishing a cause-and-effect link between them via examining this specific enzyme.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Ethics Committee of the University of Tikrit and Baghdad, College of Dentistry (**Reference No. 8**, date 1-10-2023) and conducted in accordance with institutional and international research standards. This study, which includes human individuals as participant adheres to the World Medical Association's Declaration of Helsinki. The subject's participation was officially recorded by completing a specifically tailored informed consent form. The participants in the current study gave their verbal and written agreement. The research goal was explained to the participants, and they were assured of the privacy and confidentiality of their data.

This research is a cross-sectional study that includes a sample of 150 randomly chosen people aged between 25 and 65 years old. The study collected samples from the periodontology department at the Teaching Hospital of College of Dentistry, University of Tikrit and Baghdad, as well as from the Consultation clinic for treating rheumatoid arthritis at City Medical Hospital and the Medical Rehabilitation Hospital in Tikrit. The data collection took place over three months, from October 2023 to January 2024. The rheumatoid examination involves assessing clinical indicators and doing laboratory tests to evaluate the blood levels of CBC (Complete Blood Count), ESR (Erythrocyte sedimentation Rate), CRP (C-reactive protein), and rheumatoid factors. Additionally, a radiographic examination of the bones is performed to detect any early indications of erosion. All of these tests were conducted on individuals thought to have rheumatoid arthritis (RA). Periodontal examinations will be performed routinely on all individuals with specific medical symptoms associated with the disease (rheumatoid arthritis, RA). The study included subjects who were divided into three groups: fifty individuals with periodontitis (stage II and III, grade B) according to the 2017 classification (16). Fifty patients with rheumatoid arthritis (of both genders) who also had stage II and III periodontitis (grade C), and a control group consisting of fifty subjects with a clinically healthy periodontium and good overall medical health. A salivary examination was conducted on all research participants to measure their alpha-amylase levels.

The measurement included all teeth except wisdom teeth. The study excluded individuals who were smokers, alcoholics, pregnant or lactating, and those with other systemic diseases such as diabetes, immunosuppressive disorders, or blood diseases. Additionally, patients taking antibiotics, anti-inflammatory medications, or other drugs known to affect the periodontium within the last 1.5 months were excluded.

The participants' periodontal health condition was assessed and quantified based on specific periodontal criteria, including plaque index (18), gingival index (19), bleeding on probing, probing pocket depth, and clinical attachment level (20).

Obtain consent from all subjects before doing the periodontal clinical evaluation. 3-5 milliliters of unstimulated saliva were collected in a sterile test tube by having patients spit into it for about 2-5 minutes. The saliva collection occurred between 8-11 a.m. (21). The saliva samples were maintained in tubes inside a more excellent box, with each sample numbered according to the patient. Subsequently, the saliva samples were subjected to centrifugation at a speed of 1000rpm for 10 minutes, collecting about 1 ml of supernatant saliva. This supernatant saliva was then held at a temperature of 4ºC to measure amylase levels by the liquidator colorimetric test comprising a new substrate, 2- cholo-4-nitrophenyl-maltortrioside (5 $CNPG_3$) (22).

$$5\ \mathrm{CNPG_3}(\alpha - \mathrm{amylase}) \rightarrow 3\ \mathrm{CNP} + 2\ \mathrm{CNPG_2} + 3\ \mathrm{G_3} + 2\ \mathrm{G}$$

After collecting saliva samples, a clinical examination was conducted on all teeth except the third molar. The assessment was performed using a conventional periodontal probe to assess the periodontal health status based on specified criteria, including plaque index (PLI), gingival index (GI), bleeding on probing (BOP) score 1, probing pocket depth (PPD), and clinical attachment level (CAL).

Statistical Analysis

The statistical analysis was performed using the SPSS V. 26 software program, which was used to evaluate my results. The research used descriptive statistical analysis, employing mean, mean percentage, standard deviation (SD), ANOVA test result, and Simple Pearson's correlation coefficients (r). In statistical analysis, significance levels (S) were used to determine the statistical significance of the results. Non-significant (NS) results were indicated when the p-value (P) was more than 0.05. Highly significant (HS) results were indicated when the p-value was less than or equal to 0.01. Results falling between 0.05 and 0.01 were considered moderately substantial.

RESULTS

The inquiry findings are consolidated in each of the four tables. Based on the findings presented in Table 1, the high mean value of the age parameter was 58 in rheumatoid arthritis patients with periodontitis. In contrast, the lowest mean value was 25 years old in the control group with healthy periodontium. The rheumatoid arthritis with periodontitis (stage II and III) group in Table 2 shows exclusively higher mean values for PLI, GI, BOP, PPD, and CAL, which are the clinical periodontal parameters. Their corresponding values are also as follows: 2.13, 2.53, 33.42, 5.23, and 5.13, with highly significant values appearing with the ANOVA test. Table 3 provides a detailed description of the characteristics of α-amylase in saliva. It reveals that the group with Rheumatoid arthritis (stage II and III) has a significantly higher average value of 145.13. Table (4) displays a nearly significant positive correlation between amylase levels and clinical periodontal parameters (PLI, GI, BOP score1, PPD, and CAL), with some highly substantial values observed in BOP score 1 for individuals with rheumatoid arthritis in addition to periodontitis (stages II and III2). Nevertheless, there was no significant association discovered between amylase levels and PPD in individuals with periodontitis (stage II and III, grade B)

Table (1): Descriptive data on the age characteristics of the study and control groups

Groups	**No.**	**Mean**	**±SD**
Periodontitis (stage II and III, grade B) group	50	45	0.12
Rheumatoid arthritis with periodontitis (stage II and III) group	50	58	0.33
Control group	50	25	0.42

No.=number. SD=standard deviation

Table (2): Conducting statistical analysis to assess clinical periodontal parameters among different research groups

Groups	PLI		GI		BOP score 1		PPD		CAL	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Periodontitis (stage II and III, grade B)	1.54	0.03	1.38	0.11	17.32	0.13	4.23	0.004	4.22	0.02
Rheumatoid arthritis with periodontitis (stage II and III)	2.13	0.103	2.53	0.013	33.42	0.10	5.23	0.05	5.13	0.13
Control group	0.13	0.005	-	-	-	-	-	-	-	-
ANOVA test result f-value	46.22		32.14		27.98		26.65		22.45	
P-value	0.000		0.000		0.000		0.000		0.000	
Sig.	HS		HS		HS		HS		HS	

PLI=plaque index, GI=gingival index, BOP=bleeding on the probing, CAL=clinical attachment loss, PPD=probing pocket depth, Sig.=significant, SD=standard deviation, HS=highly significant, P-value=probability

Table (3): Comparison of the study and control groups' amylase (U/L) levels statistically

Groups	α-amylase	
	Mean	±SD
Periodontitis (stage II and III, grade B)	125.02	3.65
Rheumatoid arthritis with periodontitis (stage II and III)	145.13	5.04
Control group	77.32	2.16
ANOVA test result f-value	55.285	
P-value	0.000	
Sig.	HS	

α-amylase= alpha-amylase

Table (4): Clinical periodontal characteristics in the study and control groups were correlated with amylase (U/L) levels

Groups	GI		PPD							
	r	P	r	P	r	P	r	P	r	P
Periodontitis (stage II and III, grade B)	0.33	0.02	0.23	0.04	0.36	0.05	0.43	0.06	0.86	0.013
Rheumatoid arthritis with periodontitis (stage II and III)	0.432	0.07	0.654	0.04	0.564	0.003	0.235	0.03	0.721	0.034
Control group	0.23	0.01								

P=probability, r= Simple person's correlation coefficients

DISCUSSION

The findings indicate that there is a higher average age parameter seen among persons diagnosed with Rheumatoid arthritis and periodontitis (stage II and III). This may be attributed to the fact that periodontitis is more likely to arise in older individuals. Several factors may contribute to this susceptibility, including alterations in food, heightened susceptibility to preexisting chronic systemic diseases, and age-related changes in the body's fundamental systems (23,24). Rheumatoid arthritis (RA) is a systemic autoimmune disorder that usually manifests as joint inflammation and discomfort, leading to joint damage and functional impairment. The prevalence of rheumatoid arthritis (RA) rises substantially with advancing age, and the occurrence of the illness in older individuals is not uncommon. The onset of this chronic inflammatory syndrome often occurs in individuals between the ages of 30 and 60. However, it is important to note that those after the age of 60 may also acquire this ailment (25)

The results also indicate that the mean value of clinical periodontal parameters was higher in patients with rheumatoid arthritis who had periodontitis (stage II and III) compared to other groups. This finding agrees with Rodríguez-Lozano et al. (2019) (26,27), who discovered a significant association between periodontitis and rheumatoid arthritis. Furthermore, all of the measures associated with the periodontal condition, including plaque index, bleeding on probing, probing pocket depth, and clinical attachment levels, were considerably poorer in patients with rheumatoid arthritis, where there is a clear and separate connection between periodontitis and RA.

Patients with RA experience a higher rate of periodontal disease, which is also more severe compared to those without a rheumatic inflammatory condition. Additionally, patients suffering from rheumatoid arthritis who have had more severe periodontitis, which is characterized by more clinical attachment loss, tooth loss, and a larger number and proportion of deep pockets (≥ 5mm), also tend to have more active rheumatoid disease. This association between the two conditions returns to the fact that periodontitis and rheumatoid arthritis are immunoinflammatory conditions

characterized by the invasion of leukocytes and the release of inflammatory substances, leading to the loss of bone in the periodontium and the destruction of joints, respectively. Inflammatory processes characterize both rheumatoid arthritis and periodontal disorders. Rheumatoid arthritis (RA) is characterized by chronic inflammation, in which the immune system erroneously targets the body's tissues, particularly the joints. In periodontal disorders, inflammation occurs due to bacterial infection in the gums. The overlapping inflammatory pathways may contribute to the worsening of both illnesses in afflicted people (28–30).

The result in Table (3) revealed that the higher mean value of alpha-amylase was found in rheumatoid arthritis patients who had periodontitis (stage II and III) group, and this finding agreed with (31), where they reported that the concentration of alpha-amylase in patients with RA is elevated compared to healthy individuals, suggesting its potential utility as a co-diagnostic factor, and (32)found that the research demonstrates that the quantitative assessment of alpha-amylase levels in the group with periodontitis was significantly elevated in comparison to those with gingivitis and the control group. Salivary amylase is a metalloenzyme that contains calcium and catalyzes the hydrolysis of α-glycosidic bonds between glucose residues in polysaccharides, including starch, glycogen, and dextrins. α-Amylase is not only involved in digestion but is also known to have anti-microbial characteristics. Therefore, it may play a role in the oral defense system.

The elevated levels might be attributed to the salivary glands' reaction to inflammatory conditions such as gingivitis and periodontitis, leading to an augmented production and release of specific acinar proteins such as α-amylase, therefore strengthening the oral defense system (33–35), saliva plays a crucial role in the oral immune defense system of the host, and it may indicate alterations in both health of the mouth and body as a whole. In recent decades, saliva has been used to diagnose illnesses in internal medicine (36,37). Also, the fast advancement of saliva omics has led to the recognition of saliva as a reservoir of biomarkers. Whole saliva is a reliable and noninvasive diagnostic substance that has the potential to replace blood in the

assessment, prediction, and management of certain common illnesses. The fascination with saliva is justified due to its diverse components that accurately represent the current biomarkers and plasma composition levels. In addition, saliva biomarkers include alterations in the molecular markers of RNA, DNA, and proteins found in the oral microbiota (38,39).

CONCLUSIONS

Within the limitations of the current study, it is possible to conclude that:
The salivary level of the alpha-amylase biomarker may be used as a reliable biological indicator to detect the potential development of periodontal diseases in individuals with rheumatoid arthritis. Moreover, it serves as a valuable indicator for assessing the extent of periodontal damage, predicting outcomes, and tracking the advancement of the illness in the future.

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Authors' Contribution

Irhayyim NS completed the conceptualization, data curation, formal analysis, finding acquisition, investigation, resources, methodology, project administration, supervision, software, validation, visualization, writing- original draft, writing-review, and editing.

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Ethical statement: This study was approved by the Ethics Committee of the University of Tikrit and Baghdad, College of Dentistry (**Reference No. 8**, date 1-10-2023), and conducted in accordance with institutional and international research standards.

Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declaration of Generative AI and AI-assisted technologies

During the preparation of this work, the authors used a grammar tool to improve the readability of the manuscript. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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تحليل مقارن لتركيزات إنزيم ألفا-أميليز اللعابي لدى مرضى التهاب الانسجة الداعمة للاسنان المزمن والتهاب المفاصل الروماتزمي

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قسم أمراض اللثة، كلية طب الأسنان، جامعة تكريت، تكريت، العراق

الملخص

الأهداف: تهدف هذه الدراسة إلى تقييم ومقارنة مستويات إنزيم ألفا أميليز اللعابي وحالة صحة اللثة عن طريق قياس مختلف العوامل السريرية حول اللثة مثل مؤشر البلاك(PLI) ، مؤشر اللثة(GI) ، النزيف عند المسبار(BOP) ، المسبار. عمق الجيب(PPD) ، ومستوى الارتباط السريري .(CAL) سيتم إجراء هذا التقييم على مجموعات دراسية مختلفة، وسنقوم أيضًا بفحص العلاقة بين مستويات ألفا- إنزيم الأميليز اللعابي والمعلمات السريرية حول اللثة. **المواد وطرائق العمل**: تمت ملاحظة ما مجموعه 150 مشاركًا، تتراوح أعمارهم بين 25 إلى 65 عامًا، في هذه الدراسة المقطعية. تم تقسيم المشاركين إلى ثلاث مجموعات بناءً على صحة اللثة: 50 فردًا مصابًا بالتهاب اللثة (المرحلة الثانية والثالثة، الدرجة ب)، و50 مريضًا بالتهاب المفاصل الروماتويدي (ذكور وإناث مع اصابتهم بالتهاب اللثة (المرحلة الثانية والثالثة، الدرجة ب)، و50 شخصًا يتمتعون بدواعم أسنان صحية سريريًا وصحة طبية عامة جيدة. تم تسجيل مستويات إنزيم ألفا الأميليز اللعابية GI، BOP، PPD، و CAL لجميع المشاركين. **النتائج**: أظهرت النتائج تباينات كبيرة بين مجموعات الدراسة والسيطرة من حيث التدابير السريرية حول اللثة، بما في ذلك CAL ، BOP Score 1، GI، PLI، PPD. المجموعة ذات أعلى متوسط لمستويات ألفا الأميليز هي التهاب المفاصل الروماتويدي مع التهاب اللثة (المرحلة الثانية والثالثة) (145.13)، في حين أن المجموعة الضابطة لديها أدنى متوسط لقيمة الأميليز (77.32). تبين أن العلاقة بين ألفا الأميليز ومعلمات اللثة السريرية مهمة فعليًا ومرتبطة بشكل إيجابي في المجموعات الثلاثة. **الاستنتاجات**: يعتقد أن تقييم كمية ألفا أميليز في اللعاب هو مؤشر بيولوجي ممتاز للتنبؤ بتطور أمراض اللثة لدى الأفراد الذين يعانون من التهاب المفاصل الروماتويدي. لذلك، يوفر هذا فكرة قوية عن تأثير الأمراض الجهازية على أنسجة اللثة

الكلمات المفتاحية: التهاب المفاصل الروماتزمي، التهاب الانسجة المحيطة بالسن، الفا اميليز