

Original Article

The salivary alpha-amylase concentration in patients with rheumatoid arthritis: A case-control study

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ABSTRACT

Background: Rheumatoid arthritis (RA) is one of the most common chronic inflammatory diseases. Previous studies have reported an association between stress and salivary alpha-amylase. The aim of this study was to investigate the concentration of salivary alpha-amylase in patients with RA (by elimination of stress factor).

Materials and Methods: In this case-control study, we enrolled 50 patients with RA and 48 healthy patients as the control group. The perceived stress scale questionnaire was used to evaluate scores of stress in both case and control groups, and then the participants with high scores of stress were excluded from the study. Moreover, the alpha-amylase activity kit was used for the assessment of the levels of salivary alpha-amylase. In all analyses, the significance level was considered to be < 0.05 . Finally, the obtained data were analyzed by SPSS22.

Results: Our results indicated a high score of stress in the case group (19.42 ± 5.83 units) compared with the control group (18.02 ± 6.07 units) which was not statistically significant ($P < 0.248$). Moreover, we demonstrated a high salivary alpha-amylase concentration in the case group (340.65 ± 38.04 units) compared to the control group (302.62 ± 58.72 units), which was statistically significant ($P < 0.001$). The sensitivity and specificity of this method, at >312 alpha-amylase concentrations, were 80% and 46%, respectively.

Conclusion: In general, we indicated that the alpha-amylase concentration in patients with RA is higher than the healthy controls, and can be used as a codiagnostic factor.

Key Words: Rheumatoid arthritis, salivary alpha-amylases, stress disorder

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INTRODUCTION

Immune system disorders cause abnormally low activity or overactivity of the immune system. In cases of immune system overactivity, the body attacks and damages its own tissues (autoimmune diseases).^[1] Rheumatoid arthritis (RA) is characterized by increased extra-articular inflammation, cell

proliferation, and production of autoantibodies such as rheumatoid factor and anti-citrullinated antibodies by synovial joint inflammation, which cause bone and joint destruction.^[2] In 2010, the American College of Rheumatology (ACR) and the European League Against Rheumatism introduced a new classification

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of RA diagnostic criteria. These criteria try to diagnose the disease as soon as possible.^[3,4]

Amylase is the most abundant enzyme in human saliva, which is divided into two glycosylated amylase and nonglycosylated amylase families. Recently, saliva has been considered a good biological fluid for measuring stress.^[5] Alpha-amylase, found in plants and animals, is an enzyme that hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding shorter chains thereof, dextrins, and maltose.^[6-10]

Stress is a series of reactions that can occur in response to physical, psychological, or any other stimulus that disrupts the body's homeostasis.^[11-13] The symptoms of stress usually include adrenaline production, as well as arousal, mobility, muscle contraction, and inability to concentrate.^[14] A stressful environment can accelerate cell aging, reduce life expectancy, and increase age-related diseases.^[15]

Stress has been mentioned as one of the etiological factors causing diseases. The response to physiological stress varies from person to person, and the two adrenal-pituitary axes are the systems that create it. There are several markers for measuring stress, including cortisol and salivary alpha-amylase.^[14,15,16]

Salivary alpha-amylase is one of these compounds that are released in response to sympathetic stimuli. Due to the fact that saliva is very easy to collect, alpha-amylase is easily accessible, and due to the complexity of measuring stress, it appears that alpha-amylase is an available stress marker for objectivizing stress and measuring it in various diseases and conditions.

Previous studies reported a significant association between stress and salivary alpha-amylase. Moreover, one of the important factors in RA is stress.^[15] However, no study has been conducted to evaluate the concentration of salivary alpha-amylase in patients with RA by eliminating the confounding effect of the stress factor. The purpose of the present study was to evaluate the salivary alpha-amylase concentration in patients with RA as compared to healthy controls by omitting the stress confounding effect and tries to investigate and get help from as a diagnostic aid in distinguishing the disease as soon as possible.

MATERIALS AND METHODS

Study participants

After approval by the Ethics Committee of Isfahan University of Medical Sciences (Ethical Approval No.: IR.MUI.RESEARCH.REC.1398.594), in this case-control study, 100 participants were recruited from the Department of Oral and Maxillofacial Diseases, and Al-Zahra Hospital, at Isfahan University of Medical Sciences, Iran, from December 2018 to January 2019. The case group consisted of 50 patients with RA, diagnosed and approved by a rheumatologist and based on the ACR RA diagnostic criteria, 2010.^[1,10] Moreover, the control group consisted of 50 healthy participants, matched according to age and ethnicity.

To match case and control group members in terms of environmental factors, diet, and physiological factors, the healthy participants were enrolled from the first-degree families of the patients. The participants with <18 years old, smokers, alcoholics, pregnant women, a history of salivary gland disease, oral inflammatory disease, mental disease, autoimmune diseases (except for RA), as well as the use of antidepressants, antianxiety, and contraceptive drugs were excluded from the study.

Stress evaluation

The Perceived Stress Scale (PSS) questionnaire consisting 10 questions regarding the person's feelings and thoughts in the last month was distributed among the participants. At the end of the study, people from each case and control groups were excluded from the study if they were in the upper or lower percentile of stress (20th and 80th percentile) in terms of the average score of the answer in the questionnaire, to eliminate the stress as a confounding factor.

Sample collection-laboratory analysis

The saliva samples were drawn from all participants in fasting using nonstimulating spitting saliva sampling method. The average saliva volume collected was 1.15 mL but ranged from 0.5 mL to 3.2 mL. All saliva samples were collected between 8–11 AM while participants were fasting for 1–2 h. The samples were transferred to the clinical laboratory in <24 h. Alpha-amylase activity kit (ZellBio GmbH, Ulm, Germany) was used for the measurement of the salivary alpha-amylase concentration. This kit can measure the activity of alpha-amylase by colorimetry such that the sample was incubated with

a starch-containing substrate and the reduction in blue color after the addition of iodine was compared with that in the control group. The absorbance range of alpha-amylase is 578–660 nm, according to the laboratory report.

Statistical analyses

Statistical methods of *t*-test, linear regression, Chi-square test, and Mann–Whitney test were used to analyze the data collected in the test group and compare with the control group. To measure the sensitivity and specificity of alpha-amylase concentration we used a receiver operating characteristic (ROC) curve. In all analyses, the significance level was considered to be <0.05 . Data were analyzed by IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.

RESULTS

Among 50 studied patients, 21 (42%) cases were >50 years old, 23 (46%) cases were 36–50 years old, 4 (8%) cases were 26–35 years old, and 2 (4%) cases were 18–25 years old (mean age: 46.16). In addition, among 48 healthy controls, 21 (43.8%) were >50 years old, 16 (33.3%) were 36–50 years old, 9 (18.8%) were 26–35 years old, and 2 (4.2%) were 18–25 years old (mean age: 44.93). According to the Mann–Whitney test, there was no statistically significant difference in age distribution between the patient and healthy controls ($P = 0.58$).

Among 50 patients, 16 (32%) were male and 34 (68%) were female; whereas among 48 healthy controls, 33 (68.8%) were male and 15 (31.3%) were female. According to the Chi-square test, there was a statistically significant difference in gender distribution between the patient and healthy controls ($P < 0.001$).

Our study indicated that the stress levels in the case and control groups were 19.42 ± 5.83 units and 18.02 ± 6.07 units, respectively. According to the *t*-test, there was no statistically significant difference in stress levels between the case and control groups ($P < 0.05$) [Figure 1].

We demonstrated that the salivary alpha-amylase concentration in the case and control groups was 340.65 ± 38.04 units and 302.62 ± 58.72 , respectively. According to the *t*-test, there was a statistically significant difference in salivary alpha-amylase concentration between the case and control groups ($P < 0.001$) [Figure 2].

According to the linear regression analysis, by controlling the effect of age ($P = 0.111$), stress ($P = 0.801$), and gender ($P = 0.730$), a significant difference between alpha-amylase levels in the case and control groups is seen ($P = 0.001$).

The ROC curve analysis was conducted to evaluate the diagnostic potential of alpha-amylase levels for patients with RA. We showed that the sensitivity and specificity of this assay, at >312 alpha-amylase concentrations, were 80% and 46%, respectively [Figure 3].

DISCUSSION

Salivary amylase level is more significantly increased and reacted more rapidly than cortisol by psychological stressors, suggesting that it is a better index of stress.^[17,18]

The stress level (as a confounding variable) in participants was matched between groups. In fact, matching this parameter and eliminating its confounding effect is one of the advantages of this study compared with other previous studies.

In our study, there was a statistically significant difference in gender distribution between the patient and healthy controls. The authors believe that the main reason may be the sex difference in the prevalence of autoimmune diseases, whereby females are generally more frequently affected than males.

In a study by Jung *et al.*, the association of salivary alpha-amylase concentration and stress levels in patients with systemic lupus erythematosus (SLE)

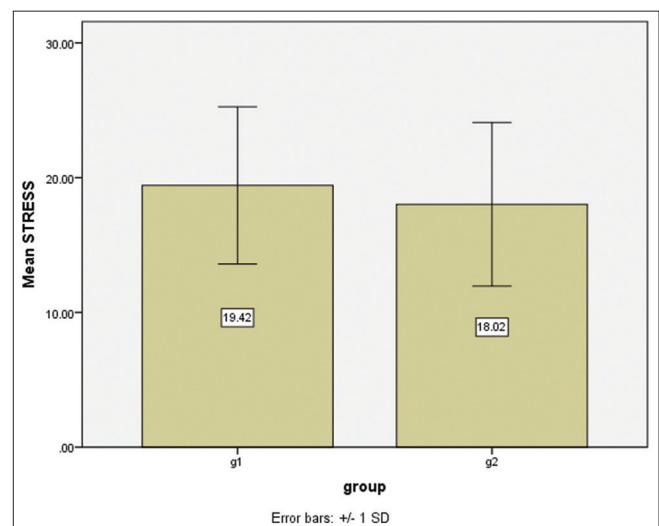


Figure 1: Mean score of anxiety (on the PSS) in patient and control groups. PSS: Perceived Stress Scale

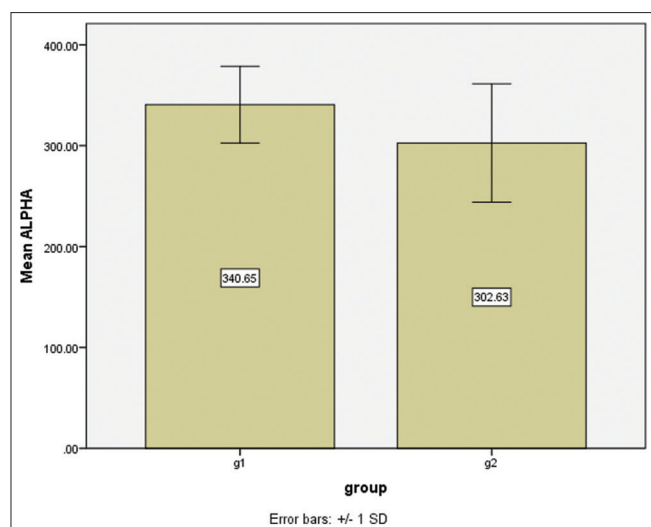


Figure 2: Mean and deviation of salivary alpha-amylase concentration (U/ml) in patient and control groups

was evaluated. The stress score was measured using PSS. They reported that alpha-amylase concentration in patients with SLE was significantly higher than in healthy controls.^[19]

Previous studies have evaluated the effects of stress on the immune system. In a study, de Brouwer *et al.* reported that high-stress level leads to reduction of interleukin-8 levels in patients with RA.^[20] In a study by Rashkova *et al.*, they reported that the concentration of salivary alpha-amylase increased at high levels of stress.^[21] In the mentioned study, individuals with further stress levels than the third quarter of stress chart were excluded to eliminate the effect of stress on salivary amylase concentration.

In another study, Kim *et al.* reported that salivary cortisol increases rapidly in high levels of stress in patients with RA. In addition, they reported that the increase in salivary alpha-amylase, due to high-stress level, was not statistically significant.^[22]

In future studies, it is suggested to use more specific and accurate tests along with the stress questionnaire and its score to verify the level of stress in the participant. Furthermore, it is recommended that the relationship between cortisol in these patients compared to controls be measured and these two factors be weighed together. Furthermore, it is suggested to select the control group from people other than the patients' relatives with a larger sample size, to provide more accurate randomization.

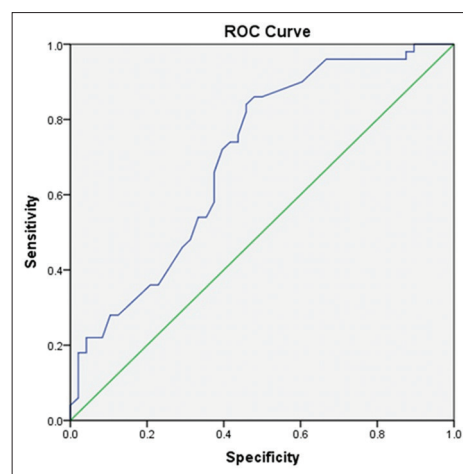


Figure 3: The ROC curve analysis. (The salivary alpha-amylase concentration in patients and controls). ROC: Receiver operating characteristics

CONCLUSION

Based on the results of this study, it was found that the level of alpha-amylase in patients with RA is higher than in the control group, and due to the sensitivity of this test, it may be considered a test along with other diagnostic modalities. This study also showed that alpha-amylase cannot be considered a screening test alone in patients with RA due to its low specificity. Furthermore, according to the results of this study, to evaluate alpha-amylase in patients with RA, the stress factor must be identified by a standard test and its confounding effect must be eliminated.

Limitations

The lack of cooperation between patients and patient companions in the study was one of the main limitations. The relatively small number of accessible RA patients was another limitation of this study, which increased the duration of the study. The authors have made every endeavor to design sex-matched controls. However, they failed to do so for several reasons including the sex difference in the prevalence of autoimmune diseases.

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Conflicts of interest

The authors of this manuscript declare that they have

no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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