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ORIGINAL ARTICLE

Salivary anti-cyclic citrullinated peptide as a screening tool for rheumatoid arthritis

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ABSTRACT

Objectives: This study aims to evaluate the sensitivity and specificity of salivary anti-cyclic citrullinated peptide 3 (anti-CCP3) for the early diagnosis of rheumatoid arthritis.

Patients and methods: Between June 2017 and April 2019, a total of 63 patients with rheumatoid arthritis (10 males, 53 females; mean age: 50.4±9.5 years; range, 27 to 74 years) and 49 healthy controls (8 males, 41 females; mean age: 49.3±9.3 years; range 27 to 67 years) were included. Salivary samples were collected by passive drooling. Anti-cyclic citrullinated peptide analyses of salivary and serum samples were performed.

Results: The mean polyclonal immunoglobulin (Ig)G-IgA anti-CCP3 salivary levels were significantly different in patients (149.2±134.2) compared to healthy controls (28.5±23.9). The mean polyclonal IgG-IgA anti-CCP3 serum levels were measured as 254.0±169.5 in patients and 3.8±3.6 in healthy individuals. The diagnostic accuracy analysis of salivary IgG-IgA anti-CCP3 results in an area under the curve (AUC) of 0.818, as well as 91.84% specificity and 61.90% sensitivity.

Conclusion: Salivary anti-CCP3 may be considered as an additional screening test for rheumatoid arthritis.

Keywords: Rheumatoid arthritis, saliva, screening.

Rheumatoid arthritis (RA) is a systemic disease which affects about 1% of the population and typically appears between 30 and 50 years old.[1] It is characterized by chronic inflammation of the synovium that is associated with joint destruction and, in some cases, disability.[2] It is thought that RA is an autoimmune disease that affects women three to four times more frequently than men.[3] A combination of genes and environment is responsible for RA. The RA-associated genes are linked with genetic factors, such as genes that express the human leukocyte antigen (HLA) major histocompatibility complex.[4] Activation of cytokines and the onset of adaptive responses may occur during the early stages of the disease.[5] Early intervention is typically accepted as critical to preventing irreversible joint damage. The importance of diagnosing RA early in the disease course cannot be overstated.[6]

The American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) Rheumatoid Arthritis Classification Criteria were published in 2010. Based on the new classification criteria, a point scoring system

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between 0 and 10 is recommended. This criterion must be applied to every individual presenting with definitive arthritis (undifferentiated inflammatory arthritis). Additional criteria are as follows: the number of joints involved, serologic abnormality, acute phase response, and duration of symptoms in the involved joints. The serological criteria include measurement of anti-citrullinated protein antibody (ACPA), such as anti-cyclic citrullinated peptide (anti-CCP), as well as definition of low positive and high positive outcomes.[7,8] Anti-CCP antibodies also called ACPAs have been found to exist early in the disease, often in the absence of clinical symptoms. Measurement of rheumatoid factor (RF) is not specific for RA, as it can be present in patients with other rheumatic or inflammatory diseases, autoimmune diseases, or chronic infections, as well as in healthy individuals. In many studies, elevated levels of anti-CCP2 antibodies were found to predict erosive disease.[9-14] Anti-CCP2 and anti-CCP3 have lately demonstrated higher sensitivity and specificity for the diagnosis of RA. Recently, anti-CCP3 has been proven to be a sensitive marker of RF-negative RA.[15,16]

Salivary proteomics has been increasingly used for the early diagnosis of diseases such as oral cancer and RA.[17,18] It has been shown that salivary immunoglobulin (Ig)A antibodies are related to a less severe RA in a previous study by Svärd et al.[19] Secretory IgA is thought to play a protective role against mucosal citrullinated antigens due to its anti-inflammatory and immunity-based aspects. The findings of Svärd et al.[20] in 2019 raised the prospect of further research in this area. In the present study, we aimed to assess the relationship between serum and salivary polyclonal IgG-IgA anti-CCP3 antibodies in RA patients.

PATIENTS AND METHODS

This case-control study was conducted at Golestan University of Medical Sciences, Department of Rheumatology between June 2017 and April 2019. Inclusion criteria were RA patients (American College of Rheumatology [ACR]/European Alliance of Associations for Rheumatology [EULAR]) with serum-positive

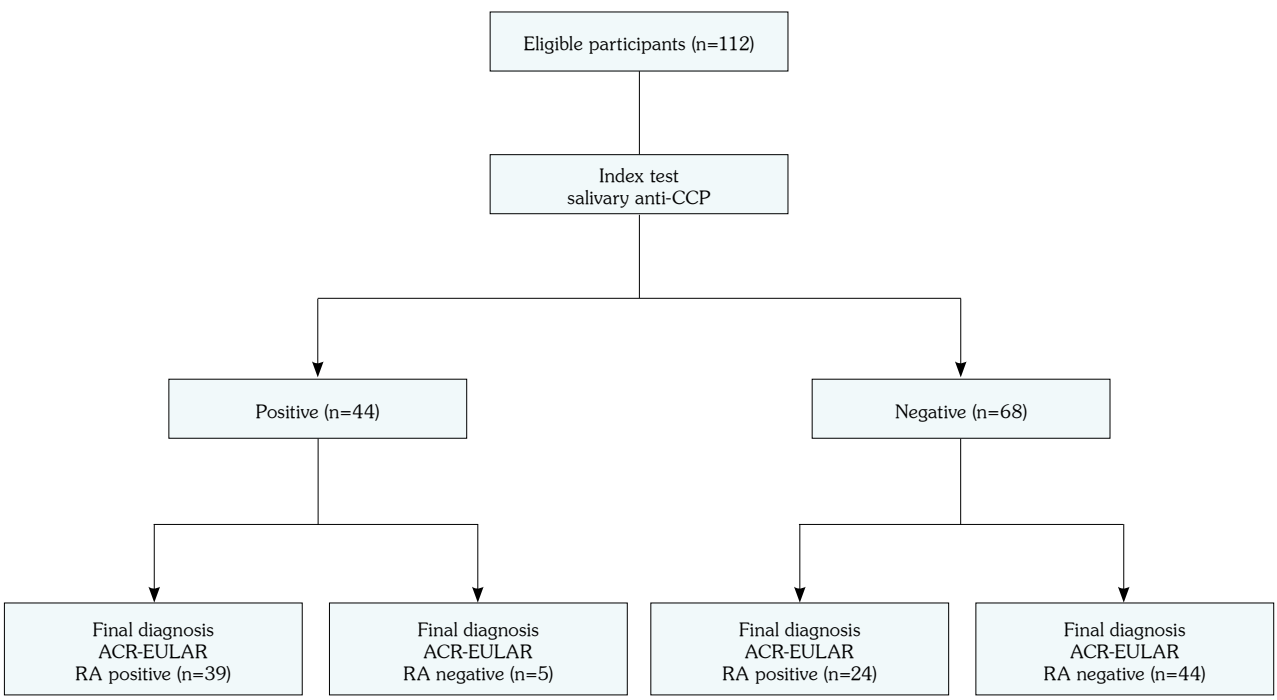

Figure 1. Study flow chart.

Anti-CCP3: Anti-cyclic citrullinated peptide; ACR: American College of Rheumatology; EULAR: European League Against Rheumatism.

IgG anti-CCP. A group of individuals who were considered to be healthy according to ACR/EULAR criteria under the supervision of a rheumatologist was also selected as the control group. A convenience sampling of 63 patients (10 males, 53 females; mean age: 50.4±9.5 years; range, 27 to 74 years) and 49 controls (8 males, 41 females; mean age: 49.3±9.3 years; range, 27 to 67 years) was carried out (Figure 1). In both groups, blood samples were collected and quantitative anti-CCP was determined. The presence of polyclonal IgG-IgA anti-CCP in saliva from individuals was examined. The participants were instructed not to eat, drink, or smoke 1 h before sampling. A salivary sample was collected by passive drooling. A volume of 5 mL of the participant's saliva was poured into a test tube embedded in ice as they bent over the front. To separate their soluble contents, specimens were immediately centrifuged for 10 min (5,000×g). Salivary specimens were, then, placed in new test tubes and then stored at -80°C for testing at the proper time. A Quanta Lite CCP 3.1 IgG/IgA enzyme-linked immunosorbent assay (ELISA) kit (Inova Diagnostics, San Diego, CA, USA) was used to analyze salivary and serum anti-CCP in this study.

Statistical analysis

Statistical analysis was performed using the SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA) and MedCalc version 13.0 software (MedCalc Software, Ostend, Belgium). Descriptive data were presented in mean ± standard deviation (SD), median (min-max) or number and frequency. To determine the normality of quantitative variables, the Kolmogorov-Smirnov test was used. In cases of normality, t-tests were used to compare means between groups. As an alternative, the Mann-Whitney U test was used to compare the mean values. The relationship between quantitative variables was determined using the Spearman correlation coefficients. The receiver operator characteristic (ROC) analysis was carried out to estimate sensitivity, specificity, and area under curve (AUC). A p value of <0.05 was considered statistically significant.

RESULTS

There was no significant difference in the age between the groups. The mean polyclonal IgG-IgA anti-CCP3 salivary levels were 149.2±134.2 in patients and 28.5±23.9 in healthy individuals (Table 1). The mean salivary IgG-IgA anti-CCP3 levels of patients and controls were significantly different (p<0.001).

The mean polyclonal IgG-IgA anti-CCP3 serum levels were 254.0±169.5 in patients and 3.8±3.6 in healthy individuals (Table 1). There was a significant difference in the mean serum IgG-IgA anti-CCP3 between patients and controls (p<0.001).

In patients, the Spearman correlation coefficient between salivary and serum levels of IgG-IgA anti-CCP3 was 0.58 (p<0.001), indicating a moderate linear relationship between serum and salivary levels. In healthy individuals, the Spearman correlation coefficient between salivary and serum levels of IgG-IgA antiCCP3 was estimated at -0.02 with no linear relationship between serum and salivary levels (p>0.05).

There was a statistically significant difference between the salivary and serum mean rank of antibody between groups of patients and controls (p<0.001).

Table 1. Mean salivary and serum levels of polyclonal IgG-IgA anti-CCP3 in patient and control groups

Sample	Patient group (n=63)	Control group (n=49)	
	Mean±SD	Mean±SD	*p*
Saliva	149.16±134.27	28.52±23.87	<0.001*
Serum	253.98±169.52	3.78±3.56	<0.001*
r	0.584	- 0.022	
p	<0.001‡	0.879‡	

Ig: Immunoglobulin; Anti-CCP3: Anti-cyclic citrullinated peptide 3; * Mann-Whitney U test; ‡ Spearman test.

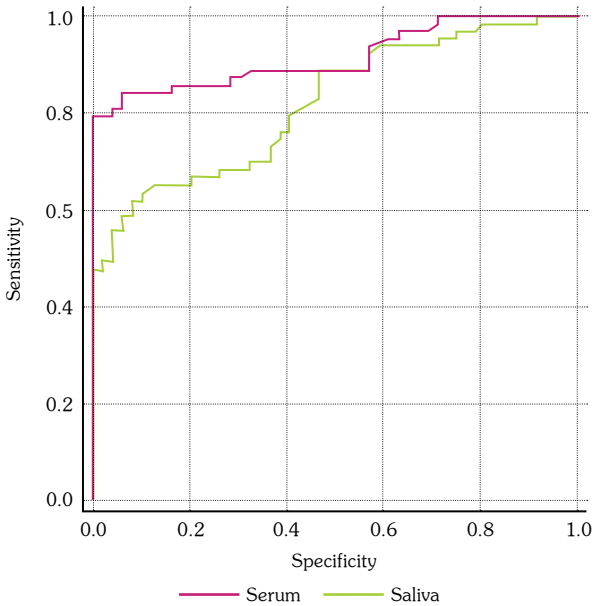

Figure 2. ROC curve for sensitivity, specificity and AUC of salivary and serum anti-CCP.

ROC: Receiver operator characteristic; AUC: Area under curve; anti-CCP: Anti-cyclic citrullinated peptide.

Using the Spearman test, the correlation coefficient between saliva and serum in men was statistically significant (p=0.029), and the same coefficient was significant in women (p<0.001). In healthy individuals, the coefficient was insignificant in both sexes (p=0.071, men) and (p=0.227, women).

Using the ROC curve analysis, 54.45 was determined to be the optimal diagnostic value for salivary cut-offs. The AUC was calculated at 0.818. Specificity and sensitivity for this value were reported as 91.84% and 61.90%, respectively. A positive predictive value of 90.70% and a negative predictive value of 65.22% were obtained.

Optimal serum cut-off point was determined at 18.55. The AUC was determined to be 0.916. The specificity and sensitivity were 100% and 79.37%, respectively. A positive predictive value of 100% and a negative predictive value of 79.03% were reported. The diagnostic accuracy stats of salivary and serum anti-CCP are shown in Figure 2.

Based on serum and saliva cut-off points, the distribution of participants was shown in Table 2. Salivary cut-off points were decided based on the smallest sum of 1-sensitivity and 1-specificity. Serum samples were also classified as negative (<20), weak positive (20-39), moderate positive (40-59), and strong positive (≥60) according to the Quanta Lite CCP 3.1 IgG/IgA ELISA kit manual guide. All 13 false-negative cases according to serum anti-CCP results were reported in the female sex.

A comparison of AUC of both salivary and serum assays would result in the salivary AUC of 81.8% and serum AUC of 91.6%. Therefore, the AUCs equality test was rejected (p=0.017). This finding would indicate that less percentage of RA patients was diagnosed through salivary assay.

DISCUSSION

Both IgG- and IgA-ACPA class antibodies are specific for detecting RA, but they are also associated with varying clinical features and different aspects of RA pathogenesis.[21] Thus, our ACPA third-generation would also include

Table 2. Saliva and serum anti-CCP test results versus disease state

	RA patients		Controls		Total	
State	n	%	n	%	n	%
Serum						
Negative	13	20.6	49	100	62	55.4
Weak positive	2	3.2	0	0	2	1.8
Strong positive	48	76.2	0	0	48	42.9
Saliva						
Negative	24	38.1	45	91.8	69	61.6
Positive	39	61.9	4	8.2	43	38.4
Total	63	100.0	49	100.0	112	100.0

Anti-CCP3: Anti-cyclic citrullinated peptide 3; RA: Rheumatoid arthritis.

those isotypes and explore the classifiable RA. It has been claimed that the last generation of ACPA (anti-CCP3 IgA-IgG) retains the specificity of ACPA2 as well as being 5 to 8% more sensitive.[13,14] According to the present study, our applied assay accelerated the number of newly identified RA cases. Meanwhile, our study indicated that the mean serum level of the antibody used was significantly different in RA patients and healthy individuals. This should have assumed the sound specificity of the third generation of antibody. Our study results are consistent with the study of Swart et al.[16] who claimed that discrimination between RA and non-RA patients was better using CCP3.

Salivary proteomics is increasingly used for the early detection of diseases such as oral cancer, Sjögren syndrome, and RA. In the recent literature, the measurement of salivary antibodies is simple and non-invasive.[17,22] A good example for this category was the study by Priyadharshini and Sathasivasubramanian[18] in which the level of anti-CCP in saliva was compared with the serum values. In their experimental group, patients' salivary anti-CCP levels were proportionally and significantly increasing with their serum anti-CCP values. Our study, having a significant p value for RA patients and also an insignificant p value for controls, is in accordance with the study conducted by the aforementioned authors. However, the present study contradicts the earlier study by Svärd et al.,[19] showing no correlation between serum and salivary anti-CCP.

According to our ROC analysis, the salivary and serum AUCs were estimated as 81.8% and 91.7%, respectively. Similar to other studies, our study confirmed the higher specificity of the corresponding antibody (in this study anti-CCP3) for the diagnosis of RA.

The fact that this antibody may be found in other autoimmune diseases (systemic sclerosis, primary biliary cirrhosis, and systemic lupus erythematosus [SLE]) also should be considered.[15]

The ROC results suggest that a lower percentage of RA patients may be diagnosed using the salivary test. An AUC over 80% would, nevertheless, allow the salivary test to be considered as a screening test. The use of saliva as a diagnostic fluid has already been studied in coeliac disease,[22] dental caries,[23] familial juvenile SLE,[17] and diabetes.[24]

Ethics Committee Approval: The study protocol was approved by the Golestan University of Medical Sciences, Ethics Committee (IR.GOUMS.REC.1396.70). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: All authors contributed equally to the article.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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