

## Evaluation of Salivary Anti-Ccp in Rheumatoid Arthritis Patients

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### Abstract:

**Aim and objective:** The aim of this study was to evaluate the levels of salivary anti-cyclic citrullinated peptide (anti-CCP) in Rheumatoid Arthritis patients. The salivary anti-CCP level was compared with the serum values.

**Materials and method:** saliva and serum samples were collected from 30 rheumatoid arthritis (group R) patients and 30 controls (group C). The samples were analysed by chemiluminescent micro particle immunoassay method. The results were compared and statistically analyzed.

**Results:** On comparing saliva and serum in controls, p-value was 0.195, was statistically insignificant. Serum and saliva of rheumatoid arthritis patients were compared and was highly significant with a p-value < 0.001.

**Conclusion:** In Group R 86.6% of patients had salivary anti-CCP levels proportionally increasing with their serum anti-CCP values with high significance. The given results of this study have shown statistically and clinically significant values and suggest that evaluation of salivary anti-CCP has a promising role in diagnosis of rheumatoid arthritis patients.

**Keywords:** anti-CCP, Diagnosis, Rheumatoid Arthritis, Saliva,

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### I. Introduction

Systemic diseases are challenging to diagnose without an appropriate evaluation of laboratory investigations. Selection of the appropriate investigations requires a profound knowledge on technique and sample selection. (1) To identify the cellular and chemical components, the most commonly used biological fluid is blood. One of the other biologic fluids that are emerging to help in diagnosis is saliva, which offers some distinctive advantages (2). Human saliva, which can be obtained by noninvasive techniques, contains many analytes of interest for screening and diagnosis. Analysis of saliva has been done for hereditary diseases, autoimmune diseases including Rheumatoid Arthritis, malignancy, infection, monitoring levels of drugs and hormones, bone turnover rate, forensic evidence, and in oral disease with relevance for systemic diseases. (3)

Rheumatoid arthritis (RA) is a systemic, autoimmune, chronic disease affecting 1% of population worldwide (4). In India, its prevalence is approximately 0.75% with more female predilection (5). It is characterized by multiple symptoms like pain, stiffness swelling and restricted movements of joints frequently involving the small joints of hands and feet. Patients with Rheumatoid arthritis will exhibit some temporomandibular joint (TMJ) involvement during the course of the disease. Early diagnosis and intervention is required to prevent irreversible joint changes.

Until 2010, the criteria used for diagnosis of RA is primarily based on 1987 American College of Rheumatology (ACR) classification criteria for Rheumatoid Arthritis (RA). A specific and sensitive marker, which is present in the early stage of disease, known as anti-cyclic citrullinated peptide (anti-CCP) has been used nowadays for a definitive diagnosis. Anti-CCP has been subsequently included in 2010 ACR criteria for Rheumatoid Arthritis. (6)

The aim of this study is to determine the levels of Anti -Cyclic Citrullinated Peptides (anti-CCP) in saliva of Rheumatoid Arthritis patients.

### II. Materials And Methods

#### II.1. Selection of patients

The study group comprised of sixty individuals, out of which thirty are controls and thirty are patients with rheumatoid arthritis. Thirty patients belonging to the control group were chosen from the Department of Oral Medicine and Radiology who reported for routine dental check up and did not have any symptoms related to arthritis. Thirty individuals who were diagnosed as cases of RA were taken as study group from Department of Rheumatology of Sri Ramachandra University.

#### II.2. Inclusion criteria

- Patients with a diagnosis of rheumatoid arthritis according to ACR/EULAR CRITERIA 2010 for rheumatoid arthritis.

**Classification criteria for RA**

(Score-based algorithm: add score of categories A–D; a score of 6/10 is needed for classification of a patient as having definite RA) (7).

CRITERIA	SCORES
A) Joint Involvement 1 large joint 2-10 large joints 1-3 small joints (with or without involvement of large joints. 4-10 small joints (with or without involvement of large joints. > 10 joints (at least 1 joint)	0 1 2 3 5
B) Serology (at least 1 test result is needed for classification) Negative RF and negative ACPA / anti-CCP Low-positive RF or low-positive ACPA/ anti-CCP High-positive RF or high-positive ACPA/ anti-CCP	0 2 3
C. Acute-phase reactants (at least 1 test result is needed for classification) Normal CRP and normal ESR Abnormal CRP or abnormal ESR	0 1
D. Duration of symptoms <6 weeks >6 weeks	0 1

**11.3exclusion Criteria**

- Patients with psoriatic arthritis
- Patients with primary Sjogrens syndrome
- Patients not willing to participate

**II.4. Sampling of Blood and Saliva**

The details of the patients were recorded in a specific case sheet proforma, which was prepared earlier. The healthy control group was labeled as Group C and their serum and salivary samples were labeled as C1 – C30. The rheumatoid arthritis patient’s group was labeled as Group R and their serum and salivary samples were labeled as R1 –R30.

**II.5. Analysis of Serum anti-CCP**

The blood samples of 4 ml were drawn from Rheumatoid Arthritis patients as well as controls using a vacutainer with a 24G needle. The blood samples were transferred immediately to the laboratory and analyzed using Abbott Architect i1000 system.

**II.6. Analysis of Salivary anti-CCP**

2ml of whole unstimulated saliva was collected by drooling method in sterile disposable plastic containers with lid. The salivary samples were transferred to sterile centrifuge tubes and immediately transported to laboratory where samples were centrifuged. These separated clear salivary fluid were immediately analyzed by chemiluminescentmicroparticle immunoassay method using the architect i1000 system.

**III. Results**

In the present study, out of 30 patients in Group R, 27 were female patients and 3 were male patients with positive serum anti-CCP. In Group C, out of 30 patients 21 female and 9 male patients were included.

In Group R,14 patients had serum anti-CCP value > 200 and 4 patients had value <0.5. Twelve patients have value more than 0.5 and below 200. In saliva, it was found that four patients had anti-CCP values <0.5 and 26 patients had more than 0.5. It is also seen that females were more predominant than males.

**Table 1: GROUP R**

Descriptive data	SERUM	SALIVA
N	30	30
Valid	30	30
Missing	0	0
Mean	142.61	2.26
Median	188.15	1.40
Mode	200.10	0.49
Std. Deviation	70.87	3.68

**Serum analysis:** The mean value for serum anti-CCP is 142.61U/ml, mode 200.10 and standard deviation is 70.87U/ml.

**Salivary analysis:** The mean value of salivary anti-CCP was found to be 2.26 U/ml, mode 0.49 and standard deviation of 3.68 U/ml.

In group C 20 patients had serum anti-CCP level less than 0.5 and 10 patients above 0.5. Salivary Anti-CCP levels were less than 0.5 in 24 patients and more than 0.5 in 6 patients.

**TABLE 2: GROUP C**

Descriptive Data		SERUM	SALIVA
N	Valid	30	30
	Missing	0	0
Mean		0.62	0.50
Median		0.49	0.49
Mode		0.49	0.49
Std. Deviation		0.38	0.042

**Serum analysis:** Mean value of serum Anti-CCP is 0.62, mode 0.49 and standard deviation is 0.38

**Salivary analysis:** Mean value of salivary Anti-CCP 0.50, mode 0.49 and standard deviation is 0.042.

On comparing saliva and serum in controls by Wilcoxon signed rank test, p-value was 0.195, which is statistically insignificant. Serum and saliva of Group R were compared and was highly significant with a p-value < 0.001.

#### IV. Discussion

Many studies in the past have evaluated the use of salivary samples instead of blood for diagnosing various systemic diseases. However, rheumatoid arthritis, which is usually investigated by evaluating anti-CCP in serum and only one study in the past, has utilized saliva, with no definite conclusion instead of serum. Hence the present study was carried out to assess the role of saliva in evaluation of anti-CCP in rheumatoid arthritis patients and to compare the value with the standard reference, which is the serum.

American College of Rheumatology / European League Against Rheumatism (ACR/EULAR) classification criteria 2010, has included anti-CCP antibodies as important criteria in the diagnosis of rheumatoid arthritis, which can be detected very early and may predict eventual development into RA when found in undifferentiated arthritis (4). Though rheumatoid factor is one of the criteria for diagnosis of RA, lack of specificity reduces its value as a diagnostic tool. According to Sharif et al specificity of rheumatoid factor is 64.7% and 89.1% for serum anti-CCP and sensitivity of RF is 85.3% and serum anti-CCP is 62.5%(8). The low sensitivity of serum anti-CCP does not exclude the disease and are more specific than rheumatoid factor (9). Among the diagnosed rheumatoid arthritis patients, anti-CCP positivity correlates with the severity of disease and these patients have more radiographic joint damage (10). Hence evaluation of anti-CCP in early stage of disease is a valid tool for diagnosis of rheumatoid arthritis. The present study has evaluated the same in serum and saliva.

In 2014, Eker et al conducted a study to evaluate the frequency of anti-CCP in rheumatoid arthritis and psoriatic arthritis patients and has revealed anti-CCP was positive in 20.6% (9/44) of psoriatic arthritis patients (11). Atzeni et al in 2008 has stated that a minority of patients with primary sjogrens syndrome is anti-CCP positive and it may be a predictor of RA in future (12). Hence patients with psoriatic arthritis and primary sjogrens syndrome are excluded from the present study.

As RA is more commonly seen in females, the present study also has 90% of patients in Group R of the same gender (5,8,13.). There are two methods employed for evaluating anti-CCP in serum, which are the Elisa and Chemiluminescence Immunoassay. But studies have shown latter method is superior to Elisa (14,15, 16). Hence the present study has used highly sensitive Chemiluminescence Immunoassay method for serum and salivary analysis.

In the present study, quantity of anti-CCP was evaluated in serum and saliva of Group C and Group R. The mean value of salivary anti-CCP in group R and group C is 2.26 and 0.5020 respectively. On comparing the mean values of both groups, p-value is 0.000, which denotes the statistical significance of salivary anti-CCP in Group R. Four patients (13.3%) in Group R had salivary anti-CCP value <0.5 and maximum value is 20.8. The salivary anti-CCP value <0.5 may be due to error in sampling technique and transport of salivary sample. In Group C 20% of patients (6 patients) had value >0.5 and 80% of patients (24 patients) had values <0.5. These six patients may be subjected for further investigations at periodic intervals for early diagnosis of rheumatoid arthritis (4,9, 17).

In the present study, the mean serum level of anti-CCP in group C was 0.62 and in group R is 142.61. On comparison of the mean value it was highly significant with a p -value of <0.001, which is in accordance with the study conducted by Khidir et al in 2013 (18).

It is also seen that salivary values in Group R are proportionally increased with serum anti-CCP levels in 86.6% (i.e.26 patients) of patients, except in 13.3%(i.e. 4 patients). On comparing serum and salivary anti-CCP values ingroup R, the p value obtained is <0.001, which indicates the high significance of using saliva as a sample in the early diagnosis of Rheumatoid Arthritis. However study conducted by Anna Svard et al (13), showed no positive correlation on comparing serum and salivary anti-CCP. In the present study, comparison of serum and salivary anti-CCP values in Group C was statistically insignificant with p-value of 0.195.

## V. Conclusion

This study evaluated the diagnostic role of saliva in rheumatoid arthritis patients by using salivary anti-CCP levels. It was performed with the objective to evaluate and compare salivary anti-CCP with serum anti-CCP levels in rheumatoid arthritis patients. In Group R 86.6% of patients had salivary anti-CCP levels proportionally increasing with their serum anti-CCP values with a highly significant p value < 0.001. Saliva has proven to be cost-effective, less-infective and a mass screening tool in diagnosis and evaluation of various systemic diseases. The given results of this study have shown statistically and clinically significant values, and suggest that evaluation of salivary anti-CCP has a promising role in diagnosis of rheumatoid arthritis patients.

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