

Research Article



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Estimation of some salivary elements in rheumatoid arthritis patients

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Abstract

**Background:** Rheumatoid arthritis (RA) is a chronic multisystemic disease which can lead to significant deformities and functional disability. RA has many oral manifestations like hyposalivation and alteration in salivary components. The aim of this study was to estimate the change in saliva composition (Iron and Total protein) in rheumatoid arthritis patients and its relation to disease activity score (DAS28). **Materials and methods:** The study design; a case control study included twenty-seven patients with rheumatoid arthritis selected as study group and twenty volunteer apparently healthy selected as control group, both groups from both genders with age range from (30-70) years. About 3 ml of unstimulated saliva samples were collected from both groups and the flow rate and PH were measured, in addition to DAS28 was counted for patients group. Estimation for the salivary total protein and salivary iron was done according to assay procedure using the spectrophotometer with a given wave length. **Results:** This study revealed no significant difference in salivary PH and iron level between RA patient and healthy control while there was significant decrease in salivary flow rate and total protein levels in RA patients. The results also show no significant difference between RA patient groups according to disease activity score for the salivary parameters. **Conclusion:** Some salivary markers decrease in Rheumatoid arthritis like total protein and flow rate. But the disease activity score has no effect on the measured salivary elements.

**Keywords:** Saliva, rheumatoid arthritis, total protein, Iron.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory musculoskeletal disease with considerable morbidity and mortality<sup>(1)</sup> and may present with extra-articular manifestations including involvement of exocrine lacrimal and salivary glands<sup>(2)</sup>. The decrease of salivary function in RA is assumed to be related to the lymphocytic infiltrate present in affected glands and manifested as a decrease of salivation and other biochemical changes<sup>(2)</sup>. The extra-articular organ involvements include the skin, eye, heart, lung, renal, nervous and gastrointestinal systems<sup>(3, 4)</sup>. In addition to oral dryness and salivary gland swelling, these

patients can also develop Sjogren's syndrome which is relatively common in 6 to 10% of patients<sup>(4 and 5)</sup>It seems appropriate to claim that both qualitative and quantitative measurements of saliva should be performed in RA patients for proper evaluation of the influence of RA on salivary function<sup>(6)</sup>.Saliva is a body fluid that contain biomarkers within its components making it an attractive diagnostic tool that could be used asan alternative to blood for measuring biomarkers<sup>(7)</sup>.The normal unstimulated salivary flow rate ranges from 0.25 to 0.35 mL/min, low value ranges from 0.1 to 0.25 mL/min, while hypo salivation

is characterized by a salivary flow rate of less than 0.1 mL/min<sup>(8)</sup>. Proteins working in cooperation with other components of saliva and have an immediate effect on oral bacteria, interfering with their ability to multiply or killing them directly<sup>(9)</sup>. Iron is utilized in nearly all aspects of cell function in living organisms, it is required for the production of energy and facilitation of cell proliferation. Immunity in living systems can be partially dependent on the availability of iron, autoimmune diseases and gout are associated with alterations in iron homeostasis supporting a participation of the metal in these injuries<sup>(10)</sup>.

Disease Activity Score 28 is included in the American College of Rheumatology 2008 Recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in RA as an outcome measure on which to base treatment decisions<sup>(11)</sup>. The aims of this study are to evaluate the level of salivary iron and total protein in rheumatoid arthritis patients, calculate salivary flow rate and PH and find the correlation between DAS28 and salivary iron level.

## Materials and Methods

### The subjects:

The study group composed of 27 patients of both genders with age range from (30-70) years. They were diagnosed clinically, by rheumatology specialists as rheumatoid arthritis depending on the seven criteria of the American Rheumatism Association with assessment of disease activity depending on Disease Activity Score 28 (DAS 28); they were attending the Baghdad Teaching Hospital. Duration of the diseases also was taken from the patients themselves with any medication or other notes about this disease. The control group consists of 20 persons with an age range (20-70) years, all the control subjects were systemically healthy and do not take any medications at least 2 weeks before sample collection.

Each patient was examined for detecting tender and swollen joints; Disease activity was assessed according to Disease Activity Score for 28 joints (DAS 28). DAS28 was calculated from the number of tender and swollen joints (28-joint count), patient's self-assessment of disease activity or Visual Analogue Scale for the patient (Patient's VAS) and ESR according to the following formula<sup>(12)</sup>:

$$\text{DAS28} = (0.56 * \text{tender joint count } 1/2) + (0.28 * \text{swollen joint count } 1/2) + (0.7 * \ln [\text{ESR}]) + (0.014 * \text{VAS}).$$

After that the patients group was divided into three groups according to DAS 28 since the level of disease activity can be interpreted as low (DAS28 < 3.2), moderate (3.2 < DAS28 < 5.1), or high (DAS28 > 5.1)<sup>(13)</sup>

### Samples collection:

About 3 ml of unstimulated saliva samples were collected from both groups (patients and control). At the same time the flow rate was calculated. The time required to collect (3 ml) of saliva from each subject was recorded. Estimation of flow rate (ml/min) was made according to this equation:

$$\text{Flow rate (FR): Volume (ml) / Time (min)}$$

After collection and disappearance of salivary foam, the pH of saliva was measured using a digital pH meter. Saliva was collected by drooling passively into screw cap, centrifuged 5 minutes at 4000 rpm, the clear supernatant was separated by micropipette and stored in eppendorf at (-20 C) till being assessed.

### Biochemical Analysis:

Chemical analysis was carried out at research lab in the Basic Science Department College of Dentistry/University of Baghdad and Poisoning Consultation Center, Medical City.

### Total Protein estimation:

Principle of the method: Proteins react in acid solution with pirogallol red and molybdate to form a colored complex, the intensity of the color formed is proportional to the protein concentration in the sample. The measurement was done according to assay procedure using spectrophotometer at 598 nm wave length<sup>(14)</sup> (Spinreact, Spain).

### Salivary Iron estimation:

Iron was determined colorimetrically using spectrophotometer at 600 nm wave length and kit of HIERRO Company, France.

### Statistical analysis

Data analysis and processing were carried using statistical package for social science SPSS version (14). The Student t-test was used to assess significant difference among means at level [P < 0.05].

**Results**

Demographic assessment of this study show in table 1, since there was female's predominance in patients

group 85.19 % while the percentage of males was 14.81%; moreover, this study found that there was 75% females and 25% males in control group.

**Table 1: Demographic assessment in rheumatoid arthritis patients group and controls (Means ± SD).**

Parameter	Rheumatoid arthritis patients	Control
Number	27	20
Age (years)	45.13±11.53	41.85±11.92
Male n. (%)	4 (14.81 %)	5 (25 %)
Female n. (%)	23 (85.19 %)	15 (75 %)

Table 2 revealed that there was significant decrease in salivary flow rate among patients group as compared to control group while there was no significant differences in salivary PH, also there was no significant differences in mean salivary iron between the two groups, furthermore there was highly significant reduction in salivary level of total protein among RA patients as compared to control group.

Also this study demonstrate that there was no significant differences in means of salivary(flow rate, PH, iron, total protein) among three groups of RA (low DAS 28, moderate DAS 28, high DAS 28) as present in table 3.

**Table 2: Descriptive statistics and Significance difference of salivary parameters for control and rheumatoid arthritis patients.**

Salivary parameters	Control Persons (Mean ± SD)	Rheumatoid Arthritis Patients (Mean ± SD)	t-test	Sig.
pH	7.1±0.18	7.2±0.29	1.000	0.500
Flow rate ml/min	0.66± 0.23	0.54±0.25	-2.397	*0.027
Iron concentration µg/dl	25.58±6.36	25.11±5.09	0.069	0.946
Total protein concentration mg/L	850.24±91.52	724.73±123.90	-3.667	**0.002

**Table 3: Difference between RA patient groups according to disease activity score for the salivary parameters**

Salivary parameters	Disease activity score DAS			t-test	Sig.
	Low (DAS28 ≤3.2) No=1	Moderate (3.2<DAS28 ≤5.1) No=16	High (DAS28>5.1) No=10		
pH	6.48	7.3±0.23	7.4±0.06	-1.700	0.188
Flow rate ml/min	0.48	0.53±0.29	0.56±0.20	-0.356	0.727
Iron concentration µg/dl	28.94	26.17±4.46	23.05±5.78	1.660	0.118
Total protein concentration mg/L	724.7	737.6±123.3	704.2±135.1	0.771	0.453

## Discussions

This study was designed to assess some salivary biomarkers in RA in comparison to healthy controls, since the present study enrolled 27 patients with RA divided into three groups depending on their DAS 28 (mild, moderate, severe). The current study found that there was significant decrease in salivary flow rate among RA patient, this result coincide with study conducted by Nagler *et al*<sup>(15)</sup>; since these authors conclude that the decrease of salivary function in RA is assumed to be related to the lymphocytic infiltrate of affected glands resulting in decreased salivation and chemical changes. Contradictory, Mignogna *et al*<sup>(16)</sup> revealed that impairment of salivary gland in the RA course; sialometry or salivary flow might not reveal any significant changes in the salivary flow rates, whereas composition of saliva may have been significantly altered by autoimmune inflammation. Meanwhile, ALosami and colleagues found that there is increase in unstimulated salivary flow rate among patients with RA on combination treatment (Methotrexate and Etanercept) showing improvements in salivary gland functions<sup>(17)</sup>. Also the present study demonstrated slightly elevation in salivary PH among patients group, however this result don't reach the significant difference. Corvo et al in 2012<sup>(18)</sup> show that no statistically significant difference in the salivary pH of individuals with secondary Sjogren s syndrome and healthy individuals, both in the non-stimulated and stimulated saliva, these findings come in agreement with results of present study. In present study the salivary pH among patients and control groups stay within physiological range pH = 6.0–7.0 (30 and 31) with slightly alkaline pH, these results are not in disagreement to expectations since the physiological salivary pH range increases as the flow rate of saliva increases and vice versa<sup>(19 and 20)</sup>.

In addition to that the current study revealed that there is significant decrease in salivary level of total protein among RA patients, this result come in agreement with study conducted by Zalweska and co-workers 2013 who stated that RA patients with xerostomia had significantly reduction in salivary level of total protein, and they denote that xerostomia in RA patients may be a harbinger of decrease of saliva production respecting quality and quantity, and may be indicative of the salivary immune system impairment of the oral cavity in RA patients with xerostomia<sup>(21)</sup>. Other study on other autoimmune disease (systemic lupus erythromatous) show that salivary total protein level was significantly higher

among patients than in the control subjects<sup>(22)</sup>. Iron plays potential role in oxidative stress, mediated injuries and pathologies e.g. rheumatoid arthritis, decades ago it was suggested that Iron may have a crucial role in progression of inflammation in rheumatoid arthritis. Indeed free radical generated by Iron can cause damage to lipids, proteins, carbohydrates and DNA. It is this destruction process that it believe to occur in rheumatoid joint<sup>(23, 24)</sup>. This study found that there was no significant differences in mean of iron in saliva of RA patients and healthy control, this study differs from study done by Ali and Al-Zubaidi<sup>(25)</sup>, since they found that there was significant reduction in level of iron in serum of RA patients, they attributed the cause to that anemia is the most common problem for people with rheumatoid arthritis as many as 60% of people with rheumatoid arthritis are anemic.

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