



Evaluation of the Salivary Oxidative Stress, and Non-Enzymatic Antioxidants Marker in Patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease that preferentially affects diarthrodial joints. Oxidative stress is elevated in RA patients implying reactive oxygen species (ROS) are possible mediators of tissue damage. Assess the level of total antioxidant capacity (TAC), malondialdehyde (MDA), and non-enzymatic antioxidants (vitamin E, and C) in the saliva of in patients with rheumatoid arthritis (RA). For the realization of our goals we included sixty rheumatoid arthritis patients (RA) (21 female and 39 male) who were fulfilling the American College of Rheumatology and European League Against Rheumatism 2010 revised criteria for classification of RA, picked randomly, at the age from 25-57 years, and 60 as age matched control group, who attended to Tikrit Teaching Hospital / Tikrit / Iraq during the period from January 2017 to March 2018. Stimulated saliva specimens were collected at the morning. TAC of saliva was evaluated by spectrophotometric assay. Statistical comparisons were performed using Student's t-test, by SPSS 13. Levels of salivary pH, flow rate, TAC, vit E and C were decreased significantly, whereas the salivary level of MDA were increased significantly in RA patients when compared with healthy controls. There was an increased oxidative stress and a low antioxidant status in patients with RA. These changes are probably due to efforts for reducing lipid peroxidation and hence to lower tissue damage.

Introduction:

Rheumatoid arthritis (RA) is a chronic autoimmune systemic disease characterized mainly by persistent inflammation in the synovial membranes

of joints, associated with migration of activated phagocytes and other leukocytes into synovial and periarticular tissue. The etiology of RA could be due to genetic and

non-genetic factors such as hormonal, environmental, and infectious factors⁽¹⁾. Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses. ROS are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they function in physiological cell processes, but prolonged existence of these ROS promotes severe tissue damage and cell death. Oxidative stress is thought to play a causative role in the pathogenesis of many inflammatory processes including rheumatoid disorders⁽²⁻⁴⁾. The saliva plays an important role in maintaining oral homeostasis, it has different defense mechanisms, such as immunological and enzyme systems, defense mechanisms against bacteria, viruses, fungi, protection of the mucosa and it also promotes its healing properties. One of the most important defense mechanisms is the antioxidant system⁽⁵⁾. This study aimed to assess the salivary TAC, MDA, Vitamin E and C in RA patients and make a comparison with healthy subjects.

Materials and Methods:

Sixty patients with RA (21 female and 39 male) followed at the Tikrit Teaching Hospital / Tikrit / Iraq were enrolled in the present study between January 2017 March 2018. All patients were confirmed to fulfill the 2010 RA classification criteria of American College of Rheumatology and European League Against Rheumatism (EULAR) ⁽⁶⁾. Total antioxidant capacity was determined by the spectrophotometric method with 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS reagent, Randox Laboratories Ltd) using autoanalyzer Konelab 20i., USA⁽⁷⁾. MDA was measured by the production of thiobarbituric acid reactive compounds (TBARS) ⁽⁸⁾. Vitamin E and Vitamin C were determined by Emer-Engle method⁽⁹⁾ and Carl A Burtis method⁽¹⁰⁾ respectively. Stimulated saliva samples were collected by spitting. 5 mL saliva of was obtained from each individual with estimating the

time of collection to obtain the flow rate. The pH of the saliva sample was measured with the help of a single electrode digital pH meter (Ottawa, Canada). Saliva were kept at 20°C and after sampling, all the samples were sent to the laboratory to be analyzed⁽¹¹⁾.

Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical analyses were performed using the Student's t-test and Pearson's correlation. Differences were considered statistically significant at the value of $p < 0.05$.

Results:

In the control group 25 were males and 35 were females. While in the study group 39 were male and 21 were females patients. The mean ages of the control and study groups were 40.1 ± 9.83 and 44.40 ± 8.13 years, respectively Table (1). Some demographic data of patients with RA and controls are listed in Table (2), The mean salivary pH, (6.98 ± 0.35), flow rate (0.52 ± 0.02), TAC (1.32 ± 0.1845 mmol/L), vitamin. E (9.71 ± 3.67 mg/L) and vitamin C (0.59 ± 0.15 mg/ dl) were significantly lower in group as compared to control group (7.3 ± 0.29 : $P < 0.000$), (0.68 ± 0.07 : $p < 0.002$), (1.8 ± 0.1417 mmol/L: $P < 0.0001$), vitamin. E (14.3 ± 1.23 mg/L: $P < 0.001$) and Vitamin C (1.06 ± 0.18 mg/ dl) respectively. While The saliva MDA level, was increased in study group (6 ± 0.48 μmol/L) in comparison with control group (3.7 ± 0.69 μmol/L: $P < 0.0001$). Table (1).

Discussion:

The level of salivary flow rate is lower in patients with RA than control group. Our findings are in the line with Nagler et al⁽¹²⁾, and Ali et al⁽¹³⁾, whom conclude that the decrease of salivary function in RA is presumed to be interrelated to the lymphocytic infiltrate of affected glands resulting in decreased salivation and chemical changes. In contrary to Mignonga et al⁽¹⁴⁾ finding, that the lymphocytic infiltration in RA disease,

might cause via cytokine secretion alteration of salivary gland function, which clinically might be expressed as increased salivary flow. Total Antioxidant Capacity (TAC), defined as the moles of oxidants neutralized by one liter of solution, is a biomarker measuring the antioxidant potential of body fluids, The present study also showed lower levels of TAC in RA patients compared to the controls, indicating decreased antioxidant activity due to oxidative stress. These results were in agreement with a study done by Anuradha et al ⁽¹⁵⁾, whereas disagree with Nagler et al ⁽¹²⁾. Salivary MDA levels are directly affected by systemic oxidative stress^(16,17). The salivary MDA level was significantly increased in the study group of the present study which reflects a high oxidative stress status among RA patients. Vitamin E serves as a chain breaking free radical trapping antioxidant, reacts with the lipid peroxide radical before the establishment of chains lipid peroxidation reactions occurs. In this process vitamin E produces tocopheroxyl free radical which is unreactive and results in forms non radical compound synthesis ⁽²⁰⁾. In the present study, the salivary level of vit. E and C

significantly were decreased in patients with RA when compared with controls due to their free radical scavenging action and to preserve the body antioxidant reserve and in normalization of vascular superoxide formation⁽²¹⁾. An epidemiological study suggested that low alpha-tocopherol status is a risk factor for RA independently of rheumatoid factor status⁽²²⁾.

Conclusions & Recommendations:

Based on the results obtained from this study, it can be concluded that the levels of MDA and other radicals are not detoxified in patients with RA due to decreased efficiency of antioxidant enzymatic and non-enzymatic mechanisms, and may act as mediators of tissue damage. Further studies with larger sample sizes and more standardized, unbiased methods are required to elucidate a causal role of vitamin E and C in RA and thus to bring about new approaches for prevention and treatment of this disease.

Table (1): Demographic characteristic of study population.

		Controls	AR patients
Age (years)		40.00±9.83	44.40±8.13
Gender	Female	35	21
	Male	25	39

Table (2): Descriptive statistics of salivary parameters for control and rheumatoid arthritis patients.

Salivary parameters	Controls	AR patients
pH	7.3±0.29	6.98±0.35**
Flow rate ml/min	0.68±0.07	0.52±0.02*
Vit. E (mg/L)	14.3±1.23	9.71±3.67*
TAC (mmol/L)	1.8± 0.1417	1.32± 0.1845**
MDA (µmol/L)	3.7±0.69	6±0.48*
Vitamin C (mg/ dl)	1.06±0.18	0.59±0.15*

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